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<p>(54) Title: METHODS OF FORMING NEURONS</p> <p>(57) Abstract</p> <p>The invention relates to novel methods of inducing non-neuronal cells to differentiate into neurons and to methods of inducing non-neuronal cells to express a neuronal subtype-specific marker.</p>		

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METHODS OF FORMING NEURONS

This is a continuing application of United States Application No. 60/096,630, filed 08/14/98, pending.

11

FIELD OF INVENTION

This invention relates to the expression of transcription factors in non-neuronal cells to induce their differentiation into neuronal cells, and more particularly, to the use the members of the neurogenin family to induce neurogenesis and the use of Phox2a or b to produce neurons which express tyrosine hydroxylase.

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BACKGROUND OF THE INVENTION

Differentiation of uncommitted neuronal precursor cells into neurons is regulated by the coordinated expression in a cascade fashion of a variety of transcription factors.

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Transcription factors are proteins that recognize and bind to specific DNA sequences located in or around chromosomal genes, and thereby regulate the expression of those genes by increasing or decreasing their rate of transcription. There are dozens of different "families" of transcription factors, members of which share a common specificity for a given DNA recognition sequence, for example, homeodomain proteins, zinc finger proteins, and basic helix-loop-helix (bHLH) proteins. Within these families there are scores of different proteins which have related but distinct structures, and different patterns of expression.

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In regards to nerve cell differentiation, transcription factors have been identified that induce precursor cells to commit to neuronal differentiation or induce committed cells to express properties shared by a specific type or subtype of neuron. For example,

1 mammalian homologs of the *Drosophila* proneural genes, called MASH1 and
NEUROGENINS (NGNs)-1, -2, and -3 (Johnson *et al.* Nature 1990. 346:858-861; Ma
et al. Cell. 1996. 87:43-52; Sommer *et al.* Cell. Neurosci. 1996. 8:221-224) are
expressed in neuronal precursors (Ma *et al.* J. Neurosci. 1997. 17:3644-3652) and are
required for commitment to a neuronal fate (Ma *et al.* Neuron. 1998 20:469-482).
6 Studies in *Xenopus* suggest that the NGNs regulate a core program of neurogenesis, that
is shared by many different classes of neurons in the central nervous system (CNS) and
peripheral nervous system (PNS) (Ma *et al.* Cell. 1996. 87:43-52; Bellefroid *et al.* Cell.
1996. 87:1191-1202). (MASH1 may play a similar, although not identical, role for
those neurogenic lineages that do not utilize NGNs (Guillemot *et al.* Cell. 1993.
11 75:463-476; Lo *et al.* Curr. Biol. 1997. 7:440-450.) In addition, paired-like
homeodomain protein, Phox2a (Valarché *et al.* Development. 1993. 119:881-896) (and
a close relative, Phox2b), is expressed by a more restricted subset of neurons in the
CNS and PNS than express the NGNs or MASH1. In particular, expression of Phox2
proteins correlates with expression of a noradrenergic neurotransmitter phenotype as
16 well as with expression of c-RET, a signal transducing receptor for Glial cell line-
Derived Neurotrophic Factor (GDNF) (Tiveron *et al.* J. Neurosci. 1996. 16:7649-7660).

It has also been reported that the NeuroD family of transcription factors are involved in
neurogenesis. In particular, it has been reported that NeuroD7 and NeuroD2 expression
21 in *Xenopus* embryos induced neurogenesis in ectodermal cells (McCormick *et al.*, Mol.
Cell Biol. 1996. 16(10):5792-5800). However, the McCormick study reports that the
ectopic neurons induced by NeuroD7 and NeuroD2 were confined to a subpopulation of
ectodermal cells, as shown, by spotty NCAM-positive staining pattern. McCormick
further reports that the apparent restricted activity of the NeuroD proteins to a subset of
26 cells derived from the ectoderm suggests that other factors may regulate their activity,
such as, the notch pathway that mediates lateral inhibition during *Drosophila*
neurogenesis.

Recent studies have explored the extent to which the differentiation of neuronal
31 precursor cells, neural crest stem cells (NCSC), to particular neuronal phenotypes can

1 be controlled by forced expression of these transcription factors. These studies have
demonstrated that forced expression of MASH1 using retroviral vectors can induce
some, but not all, NCSCs to differentiate into neurons (Lo *et al.*, Development. 1998.
125:609-620). These neurons express some markers common to all neurons, such as
neurofilament, and others that are specific to autonomic neurons in the PNS. The latter
6 include the aforementioned transcription factor Phox2a and the receptor c-RET, an
autonomic subtype marker. (Lo *et al.* Development. 1998. 125:609-620). Forced
expression of Phox2a in NCSCs, like MASH1, led to induction of c-RET; however
unlike MASH1, Phox2a was unable to promote the core program of neurogenesis (Lo *et*
al. supra). These data support the idea that the differentiation of neural stem cells to
11 particular neuronal subtypes is controlled by a combination of transcription factors,
some of which promote a core program of neurogenesis and others of which promote
expression of neuronal subtype-specific properties.

Therefore, it is an object herein to provide the compounds necessary to promote a core
16 program of neurogenesis as well as those necessary to promote expression of neuronal
subtypes. It is also an object to provide methods for inducing non-neuronal cells to
differentiate into neurons.

An additional object herein to provide methods for controlling neural stem cell
21 differentiation in order to generate neural cells of a particular phenotype in quantities
suitable for transplantation. In contrast to most other fully differentiated cells, neurons
lose their capacity to regenerate and, therefore, congenital defects, diseases or trauma to
central and peripheral nervous systems, such as, blindness, deafness, neurodegenerative
disorders, Parkinson's Disease, Huntington's Disease, and Multiple Sclerosis, and
26 damage or trauma associated with encephalitis or injury are difficult to correct.
Furthermore, tumors in neural tissues can also be very difficult to treat because of the
toxic side effects that conventional chemotherapeutic drugs may have on nervous
tissues. Surgical removal of tumors may also result in neuronal damage. Accordingly,
it is an object herein to achieve controlled differentiation of neural stem cells of the
31 CNS into dopaminergic neurons, for use in transplantation therapies of Parkinson's

1 Disease, of GABAergic striatal interneurons for therapy of Huntington's Disease, or of oligodendrocytes for therapy of Multiple Sclerosis.

A further object herein to use such neuronal differentiating agents and information provided herein for construction of test cell lines, animal models, assays for identifying
6 candidate agents which modulate neurogenesis, assays for identifying therapeutic agents, gene therapy, and differentiation of tumor cells.

SUMMARY

In accordance with the objects outlined above, the present invention provides methods
11 for inducing a non-neuronal cell to differentiate into a neuronal cell through the recombinant expression of a transcription factor that induces a core program of neurogenesis

In another aspect, the present invention provides methods for inducing the expression
16 of a neuronal subtype-specific marker in a non-neuronal cell.

In a further aspect, the invention provides expression vectors comprising transcriptional and translational control sequences operably linked to a nucleic acid encoding a member of the neurogenin family of transcription factors or a Phox2a or Phox2b
21 transcription factor, and host cells containing the expression vector(s).

In an additional aspect, the invention provides cells having an induced neuronal phenotype comprising an expression vector comprising transcriptional and translational control sequences operably linked to a nucleic acid encoding a member of the
26 neurogenin family. The invention also provides cells that have been induced to express a neuronal subtype-specific marker comprising an expression vector comprising transcriptional and translational control sequences operably linked to a nucleic acid encoding a Phox2a or Phox2b protein.

31 In a further aspect, the invention provides for identifying agents that modulate the

1 induction of a core program of neurogenesis and/or a neuronal subtype specific marker.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows the rat neurogenin-1 (NGN1) nucleic acid sequence (SEQ ID NO:1).

6 Figure 1B shows the rat NGN1 amino acid sequence (SEQ ID NO:2).

Figure 1C shows the mouse NGN1 nucleic acid sequence (SEQ ID NO:3).

Figure 1D shows the mouse NGN1 amino acid sequence (SEQ ID NO:4).

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Figure 1E shows the xenopus X-ngr-1a cDNA sequence (SEQ ID NO:5).

Figure 1F shows the xenopus X-ngr-1a amino acid sequence (SEQ ID NO:6).

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Figure 1G shows the xenopus X-ngr-1b cDNA sequence (SEQ ID NO:7).

Figure 1H shows the xenopus X-ngr-1b-amino acid sequence (SEQ ID NO:8).

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Figure 1I shows the mouse NGN2 nucleic acid (SEQ ID NO:9) and amino acid (SEQ ID NO:10) sequences.

Figure 1J shows the mouse NGN3 nucleic acid (SEQ ID NO:11) and amino acid sequences (SEQ ID NO:12).

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Figure 2A shows the mouse Phox2a nucleic acid sequence (SEQ ID NO:13).
(Valarche, *et al.* 1993. Development. 1993, 119:881-886).

Figure 2B shows the mouse Phox2a deduced amino acid sequence (SEQ ID NO:14).
The homeodomain (HD) is underlined. (Valarche, *et al.* 1993. Development. 1993,
31 119:881-886).

1 Figure 2C shows the most Phox2b nucleic acid sequence (SEQ ID NO:15). (Pattyn et al. (1997) Development 124(20):4065-4075).

Figure 2D shows the most Phox2b amino acid sequence (SEQ ID NO:16). (Pattyn et al. (1997) Development 124(20):4065-4075).

6 Figures 3A-C show the induction of neuronal differentiation in NCSCs by the bHLH transcription factor, MASH1, expressed from a MASH1-IRES-GFP encoding retrovirus. Figure 3A shows NCSCs infected with MASH1-IRES-GFP retrovirus have a process-bearing neuronal morphology. Figure 3B shows NCSCs infected with
11 MASH1-IRES-GFP retrovirus express NF160. Figure 3C shows NCSCs infected with MASH1-IRES-GFP retrovirus are identified by GFP fluorescence.

Figures 4A-C show induction of neuronal differentiation in NCSCs by the bHLH transcription factor NGN1 expressed from an NGN1-IRES-GFP retrovirus. Figure 4A
16 shows all NCSCs infected with an NGN1-IRES-GFP retrovirus have a neuronal morphology. Figure 4B shows all NCSCs infected with NGN1-IRES-GFP retrovirus stain positively with anti-NF160 antibody. Figure 4C shows all NCSCs infected with NGN1-IRES-GFP retrovirus are identified by GFP fluorescence.

21 Figures 5A-C show induction of neuronal differentiation of NCSCs grown at high density by NGN1-IRES-GFP retrovirus. Figure 5A shows the morphology of NCSCs grown at high density and infected with NGN1-IRES-GFP retrovirus. Figure 5B shows that NCSCs grown at high density and infected with NGN1-IRES-GFP retrovirus stain positively with an anti-NeuN antibody. Figure 5C shows that NCSCs grown at high
26 density and infected with NGN1-IRES-GFP retrovirus can be identified by GFP fluorescence.

Figures 6A-D show induction of a neuronal marker in cultured chick embryo fibroblasts infected with RCAS replication-competent retrovirus expressing NGN1 containing the
31 myc epitope tag. Figure 6A shows chick embryo fibroblasts infected with the RCAS

1 replication-competent retrovirus expressing NGN1 containing the myc epitope tag stain
positively with anti-myc tag antibody. Figure 6B shows chick embryo fibroblasts
infected with the RCAS replication-competent retrovirus expressing NGN1 containing
the myc epitope tag stain positively with antibody 3A10 which recognizes a
neurofilament-associated protein, NAPA-73. Figure 6C shows chick embryo
6 fibroblasts infected with the RCAS replication-competent retrovirus expressing NGN1
containing the myc epitope tag stain positively with antibody 270RMO which
recognizes NF160. Figure 6D shows chick embryo fibroblasts infected with the RCAS
replication-competent retrovirus expressing NGN1 containing the myc epitope tag stain
positively with antibody TuJ1 which recognizes beta-III tubulin. Insets of Figures
11 6B, 6C, 6D shows higher magnification of their respective figures.

Figure 7 shows the effect of added factors on TH induction by Phox2a or GFP
retroviral infected NCSCs. Abbreviations: no add=no added factor; GDNF=glial cell
16 line-derived Neurotrophic Factor; Dex=dexamethasone; F+G+D=forskolin + GDNF +
Dex.

Figure 8 shows the effect of different factors on the percentage of TH+ NCSCs in all
Phox2a retrovirus infected myc+ clones. Abbreviations: no add=no added factor;
21 GDNF=glial cell line-derived Neurotrophic Factor; Dex=dexamethasone;
F+G+D=forskolin + GDNF + Dex.

Figures 9A-D shows the effect of induced TH expression by NCSCs infected with a
retrovirus expressing myc epitope tagged Phox2a protein and cultured with added
26 factors, forskolin, FGF (fibroblast growth factor), and dexamethasone (F+G+D).
Figure 9A shows that Phox2a-myc tagged retrovirus infected NCSCs have a non-
neuronal morphology. Figure 9B shows that Phox2a-myc tagged retrovirus infected
NCSCs positively with anti-myc-tag antibody. Figure 9C shows induced TH
expression by fluorescent staining in NCSCs infected with Phox2a-myc tagged
31 retrovirus. Figure 9D shows a double exposure of Figs 9B and 9C to demonstrate that

1 many Phox2a-myc expressings NDSCs co-express TH.

Figures 10A-F show a comparison of NCSCs infected with either Phox2a-myc tagged retrovirus or GFP-myc tagged retrovirus. Figure 10A shows that NCSCs infected with Phox2a-myc tagged retrovirus and treated with forskolin have a non-neuronal morphology. Figure 10B shows that NCSCs infected with GFP-myc tagged retrovirus and treated with forskolin have a non-neuronal morphology. Figure 10C shows that NCSCs infected with Phox2a-myc tagged retrovirus and treated with forskolin stain positively with anti-myc-tag antibody. Figure 10D shows that NCSCs infected with GFP-myc tagged retrovirus and treated with forskolin stain positively with anti-myc-tag antibody. Figure 10E shows that NCSCs infected with Phox2a-myc tagged retrovirus and treated with forskolin stain positively with anti-TH antibody. Figure 10F shows that NCSCs infected with GFP-myc-tagged retrovirus and treated with forskolin do not stain with anti-TH antibody.

16 DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel methods for inducing non-neuronal cells to differentiate into neurons. The present invention also provides novel methods for inducing a non-neuronal cell to express a neuronal-subtype specific marker.

21 In a preferred embodiment, a vector encoding a transcription factor is introduced into a non-neuronal host cell. The transcription factor is operably linked to a promoter and transcription termination regulatory sequences active in the host cell. Expression of the encoded transcription factor induces the non-neuronal host cell to differentiate into a neuron. Alternatively, expression of the transcription factor induces the non-neuronal host cell to express a neuronal subtype-specific marker.

31 Accordingly, the invention provides transcription factors that can be expressed in a non-neuronal host cell and which induces the host cell to differentiate into a neuronal cell or express a neuronal sub-type specific marker. By "induce" herein is meant to cause a host cell to express at least one endogenous gene. Preferably, inducing herein

1 refers to producing a neuronal phenotype in a cell not showing such a phenotype prior
to expression of a transcription factor. By "transcription factor" as used herein is meant
a protein that regulates the transcription and expression of a gene or genes in a host cell.
In one embodiment, the transcription factor induces a core program of neurogenesis. In
another embodiment, the transcription factor induces a host cell to express a neuronal
6 subtype-specific marker. By a "core program of neurogenesis" herein is meant the
induced expression of a marker or markers common to all neurons. Examples include a
process bearing neuronal morphology, or the expression of neurofilament protein,
neuron-specific nucleoprotein, neuron-specific beta-tubulin, or NF160. It is believed
that the temporal aspect of the expression of neurogenins and the phenotype of NGN
11 knockouts contributes to the characterization of neurogenins as being the primary
initiator of neural differentiation and the induction of a cascade of genes that induce
neural differentiation. By a "neuronal subtype-specific marker or property" herein is
meant a marker or property associated with only a subset of neurons, such as, tyrosine
hydroxylase (TH) expression.

16 In a preferred embodiment, a host cell is induced to express a core program of
neurogenesis and a neuronal sub-type specific marker by the expression of a
combination of the appropriate transcription factors.

21 In one embodiment a transcription factor of the present invention includes members of
the neurogenin family. By "neurogenin" herein is meant a transcription factor, such as
neurogenin-1 (NGN1), neurogenin-2 (NGN2), or neurogenin-3 (NGN3) that is
expressed in non-neuronal cells and induces a core program of neurogenesis.

26 In another embodiment a transcription factor of the present invention includes, for
example, Phox2. By "Phox2" herein is meant a transcription factor, such as Phox2a,
that induces the expression of properties associated with a specific subtype of neuron,
such as neurons that synthesize the catecholamine family of neurotransmitters which
include dopamine, noradrenaline (norepinephrine), and adrenaline (epinephrine). For
31 example, Phox2a induces the expression of TH which is the rate-limiting enzyme in the

1 synthesis of catecholamines. In the peripheral nervous system (PNS), TH is expressed
by sympathetic autonomic neurons, and in the central nervous system (CNS) by
dopaminergic neurons of the Substantia Nigra, noradrenergic neurons of the Locus
Coeruleus, and several other groups of neurons. Other transcription factors which may
be used in accordance with the invention include POU homeodomain proteins (e.g.
6 Brn-3.0/3a); paired homeodomain proteins (e.g. DRG-11); LIM homeodomain proteins
(e.g. Isl-1, Lhx-3); Nkx-family homeodomain proteins (Dlx-1, -2, Nkx2.1, 2.2, 2.5
etc.); zinc finger protein (GATA-2, -3); bHLH proteins (eHAND, dHAND); orphan
nuclear receptors (e.g., Nurr-1); homeodomain proteins such as MNR2 or HB9.

11 By "non-neuronal cell" herein is meant any cell that is not a neuron. Therefore, a non-
neuronal cell is any cell that does not function as a conducting cell of the peripheral or
central nervous system. Accordingly, a non-neuronal cell includes uncommitted neuron
progenitors or precursor cells, for example, neural crest stems cells (NCSC) or neural
stem cells (NSC). Non-neuronal cells also includes glia (astrocytes, Schwann cells,
16 oligodendrocytes) cells that are not of a neuronal origin or lineage and include, for
example, fibroblasts. Other cells that may be used in accordance with the invention
include, for example, embryonic stem cells (ES cells); neural stem cells derived from
ES cells; mesenchymal stem cells; satellite cells; sustentacular cells; endocrine cells;
epidermal stem cells; muscle stem cells; neuroepithelial precursor (NEP) cells;
21 neuroblastoma cells and other cells types may be used.

By "neural crest stem cell" herein is meant a cell derived from the neural crest which
is characterized by having the properties (1) of self-renewal and (2) asymmetrical
division; that is, one cell divides to produce two different daughter cells with one
26 being self (renewal) and the other being a cell having a more restricted developmental
potential, as compared to the parental neural crest stem cell. The foregoing,
however, is not to be construed to mean that each cell division of a neural crest stem
cell gives rise to an asymmetrical division. It is possible that a division of a neural
crest stem cell can result only in self-renewal, in the production of more
31 developmentally restricted progeny only, or in the production of a self-renewed stem

1 cell and a cell having restricted developmental potential. The neural crest gives rise
to the peripheral nervous system (PNS).

By the term "neural stem cell" refers to a multipotent cell having properties similar
to that of a neural crest stem cell but which is not necessarily derived from the neural
6 crest. Rather, as described hereinafter, such multipotent neural stem cells can be
derived from various other tissues including neural epithelial tissue from the brain
and/or spinal cord of the adult or embryonic central nervous system or neural
epithelial tissue which may be present in tissues comprising the peripheral nervous
system. In addition, such multipotent neural stem cells may be derived from other
11 tissues such as lung, bone and the like. In a preferred embodiment, multipotent
neural stem cells are derived from the PNS, such as from the neural crest, and not
from the CNS. It is to be understood that such cells are not limited to multipotent
cells but may comprise a pluripotent cell capable of regeneration and differentiation
to different types of neurons and glia, *e.g.*, PNS and CNS neurons and glia or
16 progenitors thereof. In this regard, it should be noted that the neural crest stem cells
described herein are at least multipotent in that they are capable of self-regeneration
and differentiation to some but not all types of neurons and glia *in vitro*. Thus, a
neural crest stem cell is a multipotent neural stem cell derived from a specific tissue,
i.e., the embryonic neural tube or the sciatic nerve (Morrison et al. 1999).

21 The transcription factors of the present invention may be identified in several ways,
including, by substantial nucleic acid or amino acid sequence similarity or identity to
the sequences shown in Figures 1A-L and Figure 2A-B. Sequence similarity or identity
can be based upon the overall nucleic acid or amino acid sequence. The transcription
26 factors of the present invention have been found in vertebrates including zebrafish
(*Danio*), mice (*Mus*), rats (*Rattus*), birds (*Gallus*) and amphibians (*Xenopus*), and it is
therefore expected to be found in a number of organisms, such as zebrafish and
primates.

31 As used herein, a protein is a "neurogenin protein" if the overall similarity of the

1 protein sequence to the amino acid sequence of the neurogenin depicted herein is preferably greater than about 85%, more preferably greater than about 90% and most preferably greater than about 95%. In some embodiments the similarity will be as high as about 98-99%.

6 In addition, a neurogenin protein preferably also has a neurogenin basic-helix-loop-helix (bHLH) domain, which comprises a DNA-binding and dimerization domain (Johnson *et al.* Nature. 1990. 356:858-861).

11 As used herein, a protein is a "Phox2 protein" if the overall similarity of the protein sequence to the amino acid sequences of the Phox2a depicted herein is preferably greater than about 85%, more preferably greater than about 90% and most preferably greater than 95%. In some embodiments the similarity will be as high as about 98-99%.

16 In addition, a Phox2a protein preferably also has a homeodomain (HD). (Valarche, *et al.* 1993. Development. 1993, 119:881-886)

21 The transcription factors of the present invention may be shorter or longer than the amino acid sequences shown in the Figures 1A-L and Figure 2A-B. Thus, in a preferred embodiment, included within the definition of transcription factors of the present invention are portions or fragments of the sequences depicted herein. Generally fragments have up to about 100-150 residues, with about 15 to about 50 residues being preferred, and about 50 to about 100 residues being more preferred and about 100-150 being most preferred. Fragments of the transcription factor proteins are considered
26 transcription factor proteins if one or more of the following characteristics exist: a) they share at least one antigenic epitope; b) have at least the indicated similarity; c) and preferably have a biological activity associated with the full length sequence.
"Biological activity" includes the ability to induce a core program of neurogenesis or induce a neuronal subtype-specific phenotype.

31

1 Similarity is determined using standard techniques known in the art, including, but not
limited to, the algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the
algorithm of Needleman & Wunsch. J. Mol. Biol. 1970. 48:443, by the search for
similarity method of Pearson & Lipman. 1988. PNAS USA 85:2444, by computerized
implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the
6 Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive,
Madison, WI), or the Best Fit sequence program described by Devereux *et al.* Nucl.
Acid Res. 1984. 12:387-395.

11 In a preferred embodiment, percent identity or similarity is calculated by FastDB based
upon the following parameters: mismatch penalty of 1.0; gap penalty of 1.0; gap size
penalty of 0.33, joining penalty of 30.0. ("Current Methods in Comparison and
Analysis", Macromolecule Sequencing and Synthesis, Selected Methods and
Applications, pp. 127-149, 1998. Alan R. Liss, Inc.)

16 Another example of a useful algorithm is PILEUP. PILEUP creates a multiple
sequence alignment from a group of related sequences using progressive, pairwise
alignments. It can also plot a tree showing the clustering relationships used to create
the alignment. PILEUP uses a simplification of the progressive alignment method of
Feng and Doolittle. J. Mol. Evol. 1987. 35:351-360; the method is similar to that
21 described by Higgins and Sharp. 1989. CABIOS 5:151-153. Useful PILEUP
parameters including a default gap weight of 3.00, a default gap length weight of 0.10,
and weighted end gaps.

26 An additional example of a useful algorithm is the BLAST algorithm, described in
Altschul *et al.* J. Mol. Biol. 1990. 215:403-410 and Karlin *et al.*, PNAS USA 1993.
90:5873-5787. A particularly useful BLAST program is the WU-BLAST-2 program
which was obtained from Altschul *et al.*, Methods in Enzymology. 1996. 266: 460-480;
[[http://blast.wustl.edu/blast/ README.html](http://blast.wustl.edu/blast/README.html)]. WU-BLAST-2 uses several search
31 parameters, most of which are set to the default values. The adjustable parameters are

1 set with the following values: overlap span =1, overlap fraction = 0.125, word threshold
(T) = 11. The HSP S and HSP S2 parameters are dynamic values and are established
by the program itself depending upon the composition of the particular sequence and
composition of the particular database against which the sequence of interest is being
searched; however, the values may be adjusted to increase sensitivity.

6 An additional useful algorithm is gapped BLAST as reported by Altschul *et al.* Nucleic
Acids Res. 25:3389-3402. Gapped BLAST uses BLOSUM-62 substitution scores;
threshold T parameter set to 9; the two-hit method to trigger ungapped extensions;
charges gap lengths of k a cost of $10+k$; X_u set to 16, and X_g set to 40 for database search
11 stage and to 67 for the output stage of the algorithms. Gapped alignments are triggered
by a score corresponding to ~22 bits.

In an alternative embodiment, percent amino acid sequence identity is determined. In
percent identity calculations relative weight is not assigned to various manifestations of
16 sequence variation, such as, insertions, deletions, substitutions, *etc.* Only identities are
scored positively (+1) and all forms of sequence variation given a value of "0", which
obviates the need for a weighted scale or parameters as described above for sequence
similarity calculations. Therefore, percent identity represents a highly rigorous method
of comparing sequences.

21 Percent sequence identity can be calculated, for example, by dividing the number of
matching identical residues by the total number of residues of the "shorter" sequence in
the aligned region and multiplying by 100. The "longer" sequence is the one having the
most actual residues in the aligned region.

26 By "neurogenin nucleic acid" or "Phox2a nucleic acid" is meant, respectively, a nucleic
acid encoding a neurogenin protein or Phox2a protein, as defined herein. Nucleic acids
encoding the transcription factors of the present invention can be identified by a
number of methods as known in the art.

1 In one embodiment, the neurogenin or Phox2a nucleic acids are identified by sequence
similarity as outlined below. In the case of nucleic acids encoding the transcription
factors of the present invention the overall similarity of the nucleic acid sequence is
commensurate with the amino acid similarity of the encoded transcription factor but
takes into account the degeneracy in the genetic code and codon bias of different
6 organisms. As will be appreciated by those in the art, due to the degeneracy of the
genetic code, large numbers of nucleic acids may be made, all of which encode the
transcription factors of the present invention. Thus, having identified a particular
amino acid sequence, those skilled in the art could make any number of different
nucleic acids, by simply modifying the sequence of one or more codons in a way which
11 does not change the amino acid sequence of the encoded protein. Accordingly, the
nucleic acid sequence similarity may be either lower or higher than that of the protein
sequence. Thus the similarity of the nucleic acid sequences encoding the transcription
factors of the present invention as compared to the nucleic acid sequences of Figures
1A-L and 2A-B are preferably greater than 60%, more preferably greater than about
16 70%, particularly greater than about 75% and most preferably greater than 80%. In
some embodiments the similarity will be as high as about 90 to 95 or 98%.

Nucleic acid similarity can be determined using, for example, BLASTN (Altschul *et al.*
1990. J. Mol. Biol. 147:195-197). BLASTN uses a simple scoring system in which
21 matches count +5 and mismatches -4. To achieve computational efficiency, the default
parameters have been incorporated directly into the source code.

In another embodiment, the nucleic acid similarity is determined through hybridization
studies. Thus, for example, nucleic acids which hybridize under high stringency to the
26 nucleic acid sequences shown in the Figures (SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15) and
their complements are considered neurogenin or Phox2 genes. High stringency
conditions are known in the art; *see* for example Maniatis *et al.*, Molecular Cloning: A
Laboratory Manual, 2d Edition, 1989, and Short Protocols in Molecular Biology, ed.
Ausubel, *et al.*, Hames and Higgins, eds. *Nucleic Acid Hybridization, A Practical*
31 *Approach*, IL press, Washington, D.C., 1985; Berger and Kimmel eds. *Methods in*

1 *Enzymology, Vol. 52, Guide to Molecular Cloning Techniques*, Academic press Inc.,
New York, N.Y., 1987; and Bothwell, Yancopoulos and Alt, eds, *Methods for Cloning
and Analysis of Eukaryotic Gene*, Jones and Bartlett Publishers, Boston, Mass. 1990,
which are hereby expressly incorporated by reference in their entirety.

6 The choice of hybridization conditions will be evident to one skilled in the art and will
generally be guided by the purpose of the hybridization, the type of hybridization
(DNA-DNA, DNA-RNA, RNA-RNA, oligonucleotide-DNA *etc.*), and the level of
desired relatedness between the sequences. Methods for hybridization are well
established in the literature. For example, one of ordinary skill in the art realizes that
11 the stability of nucleic acid duplexes will decrease with an increased number and
proximity of mismatched bases; thus, the stringency of hybridization may be used to
maximize or minimize the stability of such duplexes. Hybridization stringency can be
altered by, for example, adjusting the temperature of hybridization solution; adjusting
the percentage of helix-destabilizing agents, such as, formamide, in the hybridization
16 solution; and adjusting the temperature and salt concentration of the wash solutions. In
general, the stringency of hybridization is adjusted during the post-hybridization
washes by varying the salt concentration and/or the temperature. Stringency of
hybridization may be increased, for example, by: i) increasing the percentage of
formamide in the hybridization solution; ii) increasing the temperature of the wash
21 solution; or iii) decreasing the ionic strength of the wash solution. High stringency
conditions may involve high temperature hybridization (*e.g.* 65°C-68°C in aqueous
solution containing 4-6X SSC, or 42°C in 50% formamide) combined with high
temperature (*e.g.*, 5°C-25°C below the T_m) and a low salt concentration (*e.g.*, 0.1X
SSC) washes. Reduced stringency conditions may involve lower hybridization
26 temperatures (*e.g.*, 35°C-42°C in 20-50% formamide) with intermediate temperature
(*e.g.*, 40°C-60°C) washes in a higher salt concentration (*e.g.*, 2-6X SSC). Moderate
stringency conditions, which may involve hybridization at a temperature between 50°C-
55°C and washes in 0.1X SSC, 0.1% SDS at between 50°C and 55°C, may be used
(*see* Maniatis and Ausubel, *supra*). In a preferred embodiment, nucleic acids which
31 hybridize to the nucleic acids herein have the biological activity as described herein.

1 The transcription factor encoding nucleic acids of the present invention are preferably
recombinant. As used herein, "nucleic acid" may refer to either DNA or RNA, or
molecules which contain both deoxy- and ribonucleotides. The nucleic acids include
genomic DNA, cDNA and oligonucleotides including sense and anti-sense nucleic
acids. Such nucleic acids may also contain modifications in the ribose-phosphate
6 backbone to increase stability and half life of such molecules in physiological
environments. The recombinant nucleic acids of the present invention may be double
stranded, single stranded, or contain portions of both double stranded or single stranded
sequence.

11 By the term "recombinant nucleic acid" herein is meant nucleic acid, originally formed
in vitro, in general, by the manipulation of nucleic acid by endonucleases, polymerases,
ligases, and/or recombinases to produce a form not normally found in nature.
Alternatively, a recombinant nucleic acid may be chemically synthesized according to
organic synthesis methods. Thus, a recombinant nucleic acid of the present invention
16 encodes a transcription factor that induces neurogenesis in non-neuronal cells and is
distinguished from the corresponding transcription factor-encoding nucleic acid
molecule as it exists in natural or unmodified cells.

Accordingly, a recombinant nucleic acid of the present invention can be in a linear or
21 circular form. Following introduction of a recombinant nucleic acid into a host cell the
nucleic acid can reside in a host cell as an extrachromosomal element or can be
incorporated into the genome of a host cell. A host cell can have one or multiple copies
of the recombinant nucleic acid extrachromosomally or inserted into the host cell
genome. In an alternative embodiment, a host cell may have both extrachromosomal
26 and inserted forms.

It is understood that once a recombinant nucleic acid is made and introduced into a host
cell or organism, it will replicate non-recombinantly, i.e. using the *in vivo* cellular
machinery of the host cell rather than *in vitro* manipulations; however, such nucleic
31 acids, once produced recombinantly, although subsequently replicated non-

1 recombinantly, are still considered recombinant for the purposes of the invention.

A "recombinant protein" is a protein made using recombinant techniques. A recombinant protein is distinguished from naturally occurring protein by at least one or more characteristics. For example, a recombinant protein is expressed from a
6 recombinant nucleic acid, such as an expression vector, as described below. As such, the definition of a recombinant protein includes a transcription factor protein of the present invention produced from a recombinant nucleic acid either *in vitro*, *in vivo*, or *in situ*. The recombinant protein or transcription factor can be from one organism but is expressed in a different organism or host cell. The level or degree of expression of the
11 recombinant transcription factor may be higher or lower than is normally seen. To regulate the level of expression the use of an inducible promoter may be used. In an alternative embodiment, the transcription factor may be in a form not normally found in nature, as in the addition of an epitope tag or amino acid substitutions, insertions and deletions, as discussed below.

16 In a preferred embodiment, expression of the recombinant protein is at least sufficient to induce the differentiation of the host cell into a neuron. In an alternative preferred embodiment, the expression of the recombinant protein is at least sufficient to induce the expression of a neuronal subtype-specific marker.

21 In a preferred embodiment, a recombinant nucleic acid is an expression vector. By "expression vector" herein is meant a nucleic acid that encodes and directs the synthesis of a transcription factor of the present invention. Expression of the transcription factor is effected by operably linking the sequence encoding the transcription factor to control
26 sequences.

The term "control sequences" refers to sequences necessary for the expression of an operably linked coding sequence *in vitro*, *in vivo*, or *in situ*. The control sequences that are suitable for non-neuronal cell expression, in general, include but are not limited to,
31 promoter sequences, ribosomal binding sites, transcriptional start and stop sequences,

1 translational start and stop sequences, and enhancer or activator sequences. In a
preferred embodiment, the regulatory sequences include a promoter and transcriptional
start and stop sequences.

6 Nucleic acid is "operably linked" when it is placed into a functional relationship with
another nucleic acid sequence. For example, a promoter or enhancer is operably linked
to a coding sequence if it affects the transcription of the sequence; or a ribosome
binding site is operably linked to a coding sequence if it is positioned so as to facilitate
translation. Generally, "operably linked" means that the DNA sequences being linked
are contiguous. However, enhancers do not have to be contiguous, as described below.
11 Linking the sequence encoding the transcription factor to control sequences is generally
accomplished by ligation at convenient restriction sites. If such sites do not exist, the
synthetic oligonucleotide adaptors or linkers are used in accordance with conventional
practice. Alternatively, linking can be accomplished by employing mutagenesis
techniques, PCR, recombination, organic synthesis methods, or a combination of these
16 methods, as known in the art.

A promoter is any nucleic acid sequence for all cell types including eukaryotic and
prokaryotic cells as known in the art capable of binding a RNA polymerase and
initiating the downstream (3') transcription of a coding sequence for a transcription
21 factor protein into mRNA. In a preferred embodiment, a promoter will have a
transcription initiating region, which is usually placed proximal to the 5' end of the
coding sequence, and a TATA box, using a located 25-30 base pairs upstream of the
transcription initiation site. The TATA box is thought to direct RNA polymerase to
begin RNA synthesis at the correct site. A eukaryotic promoter from a cell or virus
26 may also contain an upstream promoter element (enhancer element), typically located
within 100 to 200 base pairs upstream of the TATA box, as described below. An
upstream promoter element determines the rate at which transcription is initiated and
can act in either orientation. Of particular use as eukaryotic promoters are the
promoters from viral genes, since viral genes are often highly expressed and have a
31 broad host range. Examples include the bovine papilloma virus promoter, SV40 early

1 promoter, avian sarcoma virus LTR promoter, mouse mammary tumor virus LTR
promoter, adenovirus major late promoter, hepatitis-B virus promoter, fowlpox virus
(UK 2,211,504 published 5 July 1989), herpes simplex virus promoter, and the CMV
promoter. Examples of eukaryotic promoters from mammalian cells include, the actin
6 promoter or an immunoglobulin promoter, and heat-shock promoters, provided such
promoters are compatible with the host cell systems. Preferably, the promoter chosen is
functional in the non-neuronal cell of choice so as to control expression of the
neurogenenin or Phox2a genes.

11 In a preferred embodiment, the promoter activity is inducible or can be modulated, such
as an ecdysone-inducible promoter-enhancer combination, an estrogen-induced
promoter-enhancer combination, a tetracycline-inducible promoter-enhancer, a CMV
promoter-enhancer, an insulin gene promoter, or other cell-type specific, developmental
stage-specific, hormone-inducible, factor-inducible, or drug inducible, promoter. When
16 a hormone- or factor-inducible promoter is used, the cell must have the required
hormone or factor receptor present, either naturally or as a consequence of expression
of a co-transfected expression vector encoding such receptor. Accordingly, the host
cell must be responsive to the hormone or factor that regulates the corresponding
promoter's activity.

21 In contrast to the naturally occurring promoters, described above, alternatively the
promoters can be hybrids of two or more promoters. Hybrid or compound promoters,
which contain elements of more than one promoter, are known in the art, and are useful
in the present invention. Examples of hybrid promoters include the Tetracycline
Responsive Element/minimal immediate early promoter of cytomegalovirus. Such
26 promoters can be designed to be active in the presence or absence of tetracycline. (*see*
Clontech 98/99 Catalog, Palo Alto, CA, which is expressly incorporated by reference in
its entirety).

31 Transcription of a nucleic acid encoding the transcription factor may be increased by
inserting an enhancer sequence into the vector. Enhancers are *cis*-acting elements of

1 DNA, usually about from 10 to 300 bp, that act on a promoter to increase its
transcription. Many enhancer sequences are now known from mammalian genes
(globin, elastase, albumin, alpha-fetoprotein, and insulin). Typically, however, one will
use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on
the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter
6 enhancer, the polyoma enhancer on the late side of the replication origin, and
adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3'
to the transcription factor encoding sequence, but is preferably located at a site 5' from
the promoter.

11 Expression vectors of the present invention will also contain sequences necessary for
the termination of transcription and for stabilizing the mRNA. Such sequences are
commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic
or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as
polyadenylated fragments in the untranslated portion of the mRNA encoding the
16 transcription factor. Typically, transcription termination and polyadenylation
sequences are regulatory regions located 3' to the translation stop codon and thus,
together with the promoter elements, flank the coding sequence. The 3' terminus of the
mature mRNA is formed by site-specific post-translational cleavage and
polyadenylation. Examples of eukaryotic transcription terminator and polyadenylation
21 signals include those derived from SV40, herpes simplex virus, retroviral 3'-LTR, the
beta-globin gene, and the bovine growth hormone gene.

The expression vector may comprise additional elements. For example, the expression
vector may have two replication systems, thus allowing it to be maintained in two
26 organisms, for example, in eukaryotic cells for expression and induction of
neurogenesis and in a procaryotic host for cloning and amplification. Furthermore, for
integrating expression vectors, the expression vector contains at least one sequence
homologous to the host cell genome, and preferably two homologous sequences which
flank the expression construct (the nucleic acid encoding the transcription factor
31 operably linked to control sequences). The integrating vector may be directed to a

1 specific locus in the host cell by selecting the appropriate homologous sequence for inclusion in the vector. Constructs for integrating vectors are well known in the art.

Preferably, expression vectors will typically contain selection gene(s), also termed a selectable marker, for selection in eukaryotic and for prokaryotic cells, as needed.

6 Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, hygromycin, puromycin, bleomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

11 A further example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the transcription factor(s)-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is deficient in DHFR activity, prepared and propagated as described by Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980).

16 Still other vectors suitable for adaptation to the synthesis of transcription factors in recombinant vertebrate cell culture are described in Gething *et al.*, *Nature*, 293:620-625 (1981); Mantei *et al.*, *Nature*, 281:40-46 (1979); EP 117,060; EP 117,058; Clontech 98/99 (Palo Alto, CA), Promega 1998 (Madison, WI); and Life Technologies 97/98 (Gaithersburg, MD) catalogs.

26 Accordingly, the expression vector, for example, may be in the form of a plasmid or viral particle. Examples of plasmid expression vectors include pTarget™, pSI, pCI (Promega, Madison, WI); pSV●Sport (Life Technologies, Gaithersburg, MD); pTRE (Clontech, Palo Alto, CA). Viral expression systems include retroviruses (pBABE), adenoviruses, herpesviruses (McLean *et al. JID* 170(5):1100-1109 (1994), and togaviruses (Sindbis and Semliki Forest viruses). In a preferred embodiment, the viral vector is deficient in one or more essential genes and is replication-incompetent in target host cells. Preferred vectors are retroviral expression vectors, for example,

31

1 pBABE and others, which are preferably inducible.

In a further embodiment, the transcription factors of the present invention may also be made as a fusion protein, using techniques well known in the art. Thus, for example, the expressed transcription factor protein may be fused to a carrier protein or
6 polypeptide, for example, in order to modulate the level of expression, biological activity, or monitor the expressed factor. For example, the transcription factor can be fused to the Antp homeodomain (A. Prochiantz. (1998) *Nature Biotechnol.* 16:819-820; Derossi et al. (1998) *Trends Cell Biol.* 8:84-87).

11 To monitor expression, the transcription factor can be fused to a polypeptide that functions as an epitope tag. The epitope tag is generally placed at the amino- or carboxyl- terminus of the transcription factor. The presence of such epitope-tagged forms of the transcription factor can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the transcription factor to be
16 readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 (Field *et al.*, *Mol. Cell. Biol.*, 8:2159-2165 (1988)); the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan *et al.*, *Molecular and Cellular Biology*, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky *et al.*, *Protein Engineering*, 3(6):547-553 (1990)].
21 Other tag polypeptides include the Flag-peptide [Hopp *et al.*, *BioTechnology*, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin *et al.*, *Science*, 255:192-194 (1992)]; an
26 a-tubulin epitope peptide [Skinner *et al.*, *J. Biol. Chem.*, 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:6393-6397 (1990)].

In a further embodiment, the transcription factors of the present invention may also be
31 made as amino acid sequence variants. These variants fall into one or more of three

1 classes: substitutional, insertional or deletional variants. In one embodiment, these
variants are prepared by site specific mutagenesis of nucleotides in the DNA encoding
the transcription factor protein, using cassette, or PCR mutagenesis or other techniques
well known in the art to produce DNA encoding the variant, and thereafter expressing
the DNA in recombinant cell culture as outlined below to identify the variant with the
6 desired properties. However, variant transcription factor protein fragments having up
to about 100-150 residues may be prepared by *in vitro* synthesis using established
organic synthesis methods techniques.

11 Amino acid sequence variants are characterized by the nature of the variation, a feature
that sets them apart from naturally occurring allelic or interspecies variation of the
transcription factor amino acid sequence. The variants typically exhibit the same
qualitative biological activity as the naturally occurring analogue, although variants can
also be selected which have modified characteristics as will be more fully outlined
below.

16 In certain embodiments, when the site or region for introducing an amino acid sequence
variation is predetermined, the mutation *per se* need not be predetermined. For
example, in order to optimize the performance of a mutation at a given site, random
mutagenesis may be conducted at the target codon or region and the expressed
21 transcription factor variants screened for the optimal combination of desired activity.
Techniques for making substitution mutations at predetermined sites in DNA having a
known sequence are well known, for example, M13 primer mutagenesis and PCR
mutagenesis. Screening of the mutants is done using assays of transcription factor
protein activities. Amino acid substitutions are typically of single residues; insertions
26 usually will be on the order of from about 1 to 20 amino acids, although considerably
larger insertions may be tolerated. Deletions range from about 1 to about 20 residues,
although in some cases deletions may be much larger.

31 Substitutions, deletions, insertions or any combination thereof may be used to arrive at
a final derivative. Generally these changes are done on a few amino acids to minimize

1 the alteration of the molecule. However, larger changes may be tolerated in certain circumstances. When small alterations in the characteristics of the transcription factor protein are desired, substitutions are generally made in accordance with the following chart:

6	<u>Original Residue</u>	<u>Exemplary Substitutions</u>
	Ala	Ser
	Arg	Lys
	Asn	Gln, His
	Asp	Glu
11	Cys	Ser
	Gln	Asn
	Glu	Asp
	Gly	Pro
	His	Asn, Gln
16	Ile	Leu, Val
	Leu	Ile, Val
	Lys	Arg, Gln, Glu
	Met	Leu, Ile
	Phe	Met, Leu, Tyr
21	Ser	Thr
	Thr	Ser
	Trp	Tyr
	Tyr	Trp, Phe
	Val	Ile, Leu

26 Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those shown in Chart I. For example, substitutions may be made which more significantly affect: the structure of the polypeptide backbone in the area of the alteration, for example the alpha-helical or beta-sheet structure; the charge or hydrophobicity of the molecule at the target site; or

31 the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the polypeptide's properties are those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g.

1 lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g. glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g. phenylalanine, is substituted for (or by) one not having a side chain, e.g. glycine.

6 In one embodiment, variants exhibit the same qualitative biological activity and will elicit the same immune response as the naturally-occurring analogue. In an alternative embodiment, variants are selected to modify the characteristics of the transcription factor proteins as needed, such as, the biological activity and/or immunogenic properties, as described below.

11 In an alternative embodiment, a library of variants are generated by an entirely, non-specific, random mutagenesis method. These techniques are known in the art and do not require the selection of a specific cite or region to be altered. For example, DNA shuffling as described by Stemmer. *Nature* 370:389-391 (1994) and Stemmer. *PNAS USA* 91:10747-10751 (1994)) can be used to produce variants which are cloned,
16 expressed, and screened for a desired property. For example, the intracellular activity of the transcription factor can be increased or decreased. In addition, the number and types of genes that are regulated by the transcription factor can also be broadened or narrowed, as needed to induce expression within a host cell, as described below.

21 Also included with the definition of transcription factor variants are transcription factor proteins from other organisms, which are cloned and expressed as outlined below. Thus, probe or degenerate polymerase chain reaction (PCR) primer sequences may be used to find other related transcription factor proteins from humans or other organisms. As will be appreciated by those in the art, particularly useful probe and/or PCR primer
26 sequences include the less conserved areas and preferably, the unique areas of the nucleic acid sequence encoding the transcription factor proteins of the present invention. As is generally known in the art, preferred PCR primers are from about 15 to about 35 nucleotides in length, with from about 20 to about 30 being preferred, and may contain inosine as needed. The conditions for the PCR reaction are well known in
31 the art. It is therefore also understood that provided along with the sequences in the

1 sequences listed herein are portions of those sequences, wherein unique portions of 15
nucleotides or more are particularly preferred. The skilled artisan can routinely
synthesize or cut a nucleotide sequence to the desired length.

6 The methods of introducing the expression vectors into target host cells, are well
known in the art, and will vary with the host cell and the type of expression vector that
is used. The target host cell can be in tissue culture or, alternatively, can be in an
organism. For DNA or RNA vectors, techniques include the use of dextran-mediated
transfection, calcium phosphate precipitation, polybrene mediated transfection,
11 protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes,
and direct microinjection of the expression vector into cell or nuclei. For the case of
recombinant virus particle vectors, entry into a host cell is mediated via attachment of
the virus particle to the host cell followed by penetration of the host cell membrane and
introduction of the viral nucleocapsid into the host cell. The mechanism of entry will
vary according to the type of virus vector being used but generally will follow the
16 mechanisms of entry of wild-type virus.

Transformed host cells of the present invention find a variety of *in vitro* uses, for
example: i) as convenient sources of neuronal and other growth factors, ii) in transient
and continuous culture for screening drugs or compounds that are either antagonists or
21 protagonists of neural differentiation as it relates to normal differentiation and
development, neural repair, and tumor development, iii) as sources of recombinantly
expressed neurogenins and/or Phox2a proteins for use as an antigen in preparing
monoclonal and polyclonal antibodies useful in diagnostic assays, iv) in transient and
continuous cultures for screening for compounds capable of increasing or decreasing
26 the activity of neurogenin and/or Phox2a, vi) for use in transplantation, as described
below and, vii) *in vivo* delivery of the genes into adult neural stem cells to induce
neurogenesis *in vivo*.

31 For expression in host cells, specific conditions may vary with the cell type being used
and the desired neuron or neuronal subtype-specific marker to be produced. The

1 transcription factors of the present invention are produced by culturing a host cell
transformed with an expression vector containing nucleic acid encoding a neurogenin
or Phox2a protein. under the appropriate conditions cause expression of the encoded
factor. The conditions appropriate for the specific transcription factor(s) expression
will vary with the choice of the expression vector and the host cell, and will be easily
6 ascertained by one skilled in the art through routine experimentation. For example, the
use of constitutive promoters in the expression vector will require optimizing the
growth and proliferation of the host cell, while the use of an inducible promoter
requires the appropriate growth conditions for induction. In addition, in some
embodiments, the timing of the harvest is important.

11 In a preferred embodiment, expression of the transcription factors of the present
invention in a non-neuronal host cell induces the cell to differentiate into a neuron. In
an another embodiment, expression of the transcription factors of the present invention
induce the cell to express and neuronal subtype-specific marker. In yet another
16 embodiment, expression of the transcription factors of the present invention in a non-
neuronal host cell induces the cell to differentiate into a neuron and to express a
neuronal subtype-specific marker. Appropriate host cells for the induction of a
neuronal phenotype/expression include, for example, neural stem cells, neural crest
stem cells, and cells of a non-neuronal origin or lineage, such as, fibroblasts or
21 epithelial cells or as described *supra*. Especially preferred cells are embryonic stem
cells.

In a preferred embodiment, expression of neurogenin in non-neuronal cells and
uncommitted neuronal precursor cells, such as, neural stem cells or neural crest stem
26 cells or fibroblasts induces a core program of neurogenesis associated with the
commitment of a cell to differentiate into a neuron cell. The core program of
neurogenesis include a number of markers common to all neurons. Examples of these
markers include adoption of a neuronal morphology, or expression of neurofilament,
neuron-specific nucleoprotein, neuron-specific beta-tubulin, NF160, NeuN, SCG10,
31 neuron-specific enolase, PGP9.5, hi-PSA NCAM, synapsin I.

1 In another preferred embodiment, expression of Phox2a in non-neuronal cells and
uncommitted neuronal precursor cells induces the expression of properties associated
with specific neuronal subtype, for example, neurons that synthesize catecholamine
neurotransmitters which include dopamine, noradrenaline, and adrenaline. Phox2a
preferably induces the expression of tyrosine hydrolase (TH) which is the rate-limiting
6 enzyme in the synthesis of catecholamines. In the PNS, TH is expressed by
sympathetic autonomic neurons, and, noradrenergic neurons of the Locus Coeruleus,
and several other groups of neurons. It is therefore desirable to be able to control the
differentiation of neurons that express TH, from neural stem cells.

11 In yet another preferred embodiment, expression of neurogenin and Phox2a in non-
neuronal cells induces the expression of both a core program of neurogenesis and
properties associated with a neurons that synthesize catecholamines.

The cells of the present invention also find a variety of *in vivo* uses, for example, for
16 transplantation at sites of neuronal disfunction. For example, cells are transformed *in*
vitro and are transplanted into an organism. In a preferred embodiment, the
transplanted host cells replace or enhance functions of neurons that communicate via
electrical or chemical synapses. Examples of neurons that communicate via chemical
synapses include, for example, peptidergic, serotonergic, noradrenergic, cholinergic,
21 glutamatergic, GABAergic, dopaminergic, and noradrenergic neurons.

In a preferred embodiment the transplantation is autologous but alternatively can be
heterologous or a xenographic transplant. For other than autologous transplantation,
immune suppressors or modifiers are preferably employed, as known in the art, to
26 prevent destruction of the transplanted cells or tissue by a host verses graft response.

The transformed cells are transplanted in a quantity to be therapeutically effective. A
therapeutically effective quantity or dosage refers to a dosage adequate to ameliorate
symptoms or signs of the disease without producing unacceptable toxicity to the
31 patient. In general, an effective quantity of transplanted cell is that which provides

1 either subjective relief of symptoms or an objectively identifiable improvement as
noted by the clinician or other qualified observer.

6 The dosage or quantity of transplanted cells used in accordance with this invention
varies depending on the cell and the condition being treated. The age, weight, and
clinical condition of the recipient patient, and the experience and judgment of the
clinician, practitioner, or veterinarian administering the therapy are among the factors
affecting the selected dosage. Other factors include the patient's medical history, the
severity of the disease process, and the potency of the particular transplanted cells.

11 The host cells can be transplanted to either the central or peripheral nervous system.
The central nervous system (CNS) includes, for example, the cortex, hippocampus,
septum, striatum, the cerebrum, cerebellum, pons, medulla oblongata, neural tissues of
the pituitary gland, the spinal cord etc. The peripheral nervous system (PNS) includes
all neural tissue that is not a component of the CNS.

16 In another aspect of the invention, the expression vectors are introduced into cells *in*
vivo. Accordingly, induction of a core program of neurogenesis or a neuronal subtype-
specific marker occurs *in vivo* in cells containing an expression vector of the present
invention. The compositions for administration will preferably comprise a solution of
21 the expression vector dissolved or suspended in a pharmaceutically acceptable carrier.
The type of pharmaceutically acceptable carrier will be directed, in part, by the type of
expression vector that is employed, for example, a nucleic acid vector or a viral vector.
A variety of carriers can be used, e.g., buffered saline containing suitable emulsifiers,
and the like. Methods of producing liposomes and complexing or encapsulating
26 compounds therein are well known to those of skill in the art (*see, e.g.,* Debs and Zhu
(1993) WO 93/24640; Mannino and Gould-Fogerite (1988) *BioTechniques* 6(7):
682-691; Rose U.S. Pat No. 5,279,833; Brigham (1991) WO 91/06309; and Felgner *et*
al. (1987) *Proc. Natl. Acad. Sci. USA* 84: 7413-7414).

31 In a preferred embodiment, it is desirable to package, complex, or otherwise combine

1 the expression vector with a delivery vehicle that preferably increases cellular uptake
and/or half-life. A wide variety of suitable vehicles are well known to those of skill in
the art. Thus, for example, the expression vector can be complexed with, or
encapsulated within, a charged lipid to form a net neutral composition. This will reduce
clearance by the reticuloendothelial system and enhance cellular uptake.

6 In another embodiment, the expression vector can be encapsulated within or complexed
with microparticles which can be recognized and phagocytosed by a target cell thereby
facilitating entry of the expression vector into the cell. Other methods of facilitating
entry include the use fusion proteins, protein complexes, and masking charged groups
11 with reversible chemical modification or counterions. Viral vectors, such as,
adenovirus, retroviruses (e.g. lentivirus), herpesviruses (e.g. herpes simplex virus),
togaviruses (e.g., Sindbis virus), also can be used.

For certain of the therapeutic uses of the subject expression vectors, particularly
16 peripheral uses such as for induction of neurogenesis in the peripheral nervous system,
direct (e.g., topical or injected) administration of the expression vector will be
appropriate, according to the type of expression vector that is employed. Accordingly,
the subject expression vector, alone or in combination with a delivery vehicle may be
conveniently formulated for administration with a biologically acceptable medium,
21 such as water, buffered saline, polyol (for example, glycerol, propylene glycol, liquid
polyethylene glycol and the like) or suitable mixtures thereof. In preferred
embodiments, the expression vector is dispersed in lipid formulations, such as micelles,
which closely resemble the lipid composition of natural cell membranes to which the
expression vector is to be delivered.

26 As indicated above, the expression vectors are preferably combined with a
pharmaceutically acceptable carrier for *in vivo* administration. Pharmaceutically
acceptable carriers (excipients) can contain a physiologically acceptable compound that
acts, for example, to solubilize the composition, and/or to stabilize the composition,
31 and/or to increase or decrease the absorption of the agent. Physiologically acceptable

1 compounds can include, for example, carbohydrates, such as glucose, sucrose, or
dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low
and/or high molecular weight proteins, compositions that reduce the clearance or
hydrolysis of the expression vectors, or excipients or other stabilizers and/or buffers.
Other physiologically acceptable compounds include wetting agents, emulsifying
6 agents, dispersing agents or preservatives which are particularly useful for preventing
the growth or action of microorganisms.

The expression vector pharmacological compositions are preferably sterile and
generally free of undesirable matter. These compositions may be sterilized by
11 conventional, well known sterilization techniques. The compositions may contain
pharmaceutically acceptable auxiliary substances as required to approximate
physiological conditions such as pH adjusting and buffering agents, toxicity adjusting
agents and the like, for example, sodium acetate, sodium chloride, potassium chloride,
calcium chloride, sodium lactate and the like.

16 The concentration of expression vector in these formulations can vary widely, and will
be selected primarily based on fluid volumes, viscosities, body weight and the like in
accordance with the particular mode of administration selected and the patient's needs.

21 Where the expression vector is used in a therapeutic context, (*e.g.*, in the treatment of a
condition characterized by neuronal dysfunction or deficiency), a therapeutically
effective quantity of expression vector is employed in treatment. A therapeutically
effective quantity or dosage refers to a dosage adequate to ameliorate symptoms or
signs of the disease without producing unacceptable toxicity to the patient. In general,
26 an effective amount of the compound is that which provides either subjective relief of
symptoms or an objectively identifiable improvement as noted by the clinician or other
qualified observer.

31 The dosage of expression vector compositions used in accordance with this invention
varies depending on the compound and the condition being treated. The age, weight,

1 and clinical condition of the recipient patient, and the experience and judgment of the
clinician, practitioner, or veterinarian administering the therapy are among the factors
affecting the selected dosage. Other factors include the route of administration, the
patient's medical history, the severity of the disease process, and the potency of the
particular compound.

6 Representative patient populations that may benefit from transplantation include:
patients with hearing or vision loss due to optical or auditory nerve damage, patients
with central or peripheral nerve damage and loss of motor or sensory neural activity,
patients with brain or spinal cord damage, patients with neurodegenerative disease or
11 disorders. The damage may be the result of trauma and can be induced by injury,
accident, stroke (infarction, ischemia, hypoxia) or medical treatment, for example,
surgery, or may represent a congenital birth defect, for example, paralysis, blindness, or
deafness. The damage may also be the result of an autoimmune disease or the sequelae
of an infectious disease, for example, meningitis, encephalitis, human
16 immunodeficiency virus, and prions.

Representative neurodegenerative diseases and disorders that are treated or ameliorated
by the transformed host cells of the present invention include, for example, Alzheimer's
Disease, Amyotrophic Lateral Sclerosis (ALS), Huntington's Disease (HD), Multiple
21 Sclerosis (MS), Parkinson's Disease (PS), and Epilepsy.

The transformed host cells of the present invention also find use in the identification of
compounds or candidate bioactive agents, such as, proteins including polypeptides and
oligopeptides, lipids, carbohydrates, nucleic acids, including oligonucleic acid and
26 antisense nucleic acids, small organic molecules, inorganic molecules, steroids, *etc.* that
modulate the activity of the transcription factors of the present invention. Thus, in one
embodiment, this invention provides methods of identifying transcription factor
modulators that specifically block or enhance transcription factor activity.

31 The methods involve screening the "candidate compound's" ability to modulate

1 induction of a core program of neurogenesis and/or a neuronal subtype-specific
property in host cells transformed with an expression vector of the present invention.
The host cell can be a cell in culture, in an organism, or, alternatively transgenic
animals may be used.

6 Screens may be designed to first find candidate agents that can bind to transcription
factor proteins, and then these agents may be used in assays that evaluate the ability of
the candidate agent to modulate transcription factor protein activity. Thus, as will be
appreciated by those in the art, there are a number of different assays which may be run;
binding assays and biological activity assays.

11 Thus, in a preferred embodiment, the methods comprise combining transcription factor
protein and a candidate bioactive agent, and determining the binding of the candidate
agent to the transcription factor protein. Preferred embodiments utilize the
transcription factor proteins as described herein but , although other transcription factor
16 proteins may also be used, including rodents (mice, rats, hamsters, guinea pigs, etc.),
farm animals (cows, sheep, pigs, horses, etc.) and primates (humans). These latter
embodiments may be preferred in the development of animal models of human disease.
In some embodiments, as outlined herein, variant or derivative transcription factor
proteins may be used, as outlined above.

21 The term "candidate bioactive agent" or "exogeneous compound" as used herein
describes any molecule, e.g., protein, polypeptide, oligopeptide, lipids, carbohydrates,
nucleic acids, oligonucleic acid, including antisense nucleic acids, small organic
molecules, inorganic molecules, steroids, *etc.*, with the capability of directly or
26 indirectly altering the biological activity of transcription factor protein. Generally a
plurality of assay mixtures are run in parallel with different agent concentrations to
obtain a differential response to the various concentrations. Typically, one of these
concentrations serves as a negative control, i.e., at zero concentration or below the level
of detection.

31

1 Modulation of transcription factor biological activity is indicated at the first detectable
level. A change in activity, which can be an increase or decrease, is preferably a
change of at least 20% to 50%, more preferably by at least 50% to 75%, more
preferably at least 75% to 100%, and more preferably 150% to 200%, and most
preferably is a change of at least 2 to 10 fold compared to a control.

6 Nucleic acids which encode transcription factor protein or its modified or variant forms
can also be used to generate either transgenic animals or "knock out" animals which, in
turn, are useful in the development and screening of therapeutically useful compounds
or reagents. A transgenic animal (e.g., a mouse or rat) is an animal having cells that
11 contain a transgene, which transgene was introduced into the animal or an ancestor of
the animal at a prenatal, e.g., an embryonic stage. A transgene is a DNA which is
integrated into the genome of a cell from which a transgenic animal develops. In one
embodiment, cDNA encoding a transcription factor protein can be used to clone
genomic DNA encoding an transcription factor protein in accordance with established
16 techniques and the genomic sequences used to generate transgenic animals that contain
cells which express the desired DNA from a transgene. Methods for generating
transgenic animals, particularly animals such as mice or rats, have become conventional
in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009.
Typically, particular cells would be targeted for the transcription factor protein
21 transgene incorporation with tissue-specific enhancers. Transgenic animals that include
a copy of a transgene encoding transcription factor protein introduced into the germ line
of the animal at an embryonic stage can be used to examine the effect of increased
expression of the desired nucleic acid. Such animals can be used as tester animals for
reagents thought to confer protection from, for example, pathological conditions
26 associated with its overexpression. In accordance with this facet of the invention, an
animal is treated with the reagent and a reduced incidence of the pathological condition,
compared to untreated animals bearing the transgene, would indicate a potential
therapeutic intervention for the pathological condition.

31 Alternatively, a transcription factor protein "knock out" animal which has at least one

1 defective, deleted, or altered allele encoding a transcription factor protein as a result of
homologous recombination between the endogenous gene encoding a transcription
factor protein and altered genomic DNA encoding a transcription factor protein
introduced into an embryonic cell of the animal. For example, cDNA encoding an
transcription factor protein can be used to clone genomic DNA encoding a transcription
6 factor protein in accordance with established techniques. A portion of the genomic
DNA encoding a transcription factor protein can be deleted or replaced with another
gene, such as a gene encoding a selectable marker which can be used to monitor
integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and
3' ends) are included in the vector [see e.g., Thomas and Capecchi, Cell, 51:503 (1987)
11 for a description of homologous recombination vectors]. The vector is introduced into
an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced
DNA has homologously recombined with the endogenous DNA are selected [see e.g.,
Li et al., Cell, 69:915 (1992)]. The selected cells are then injected into a blastocyst of
an animal (e.g., a mouse or rat) to form aggregation chimeras [see e.g., Bradley, in
16 *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson,
ed. (IL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a
suitable pseudopregnant female foster animal and the embryo brought to term to create
a "knock out" animal. Progeny harboring the homologously recombined DNA in their
germ cells can be identified by standard techniques and used to breed animals in which
21 all cells of the animal contain the homologously recombined DNA or in which both
alleles are defective, deleted, or altered. Knockout animals can be characterized for
instance, for their life-expectency and cause of death, their ability to defend against
certain pathological conditions and for their development of pathological conditions
due to absence of the transcription factor protein polypeptide. For example, knockouts
26 in NGN-1, -2, MASH1, Phox 2a, Phox 2b, and combinations thereof are made, for
example, in mice. It is understood that cell based knock-out or "knock-in" systems can
also be made and utilized in accordance with the present disclosure.

It is understood that the models described herein can be varied. For example, "knock-
31 in" models can be formed, or the models can be cell-based rather than animal models.

1 The following examples serve to more fully describe the manner of using the above-
described invention, as well as to set forth the best modes contemplated for carrying out
various aspects of the invention. It is understood that these examples in no way serve to
limit the true scope of this invention, but rather are presented for illustrative purposes.
All cited/referenced patents, patent applications, publications and references cited
6 therein are expressly incorporated by reference in their entirety.

EXAMPLES

Example 1

Induction of neuronal differentiation in neural crest stem cells by forced expression of 11 NGN1.

A retroviral vector harboring an *ngn1* cDNA was constructed so that the NGN1 coding
sequence is followed by an internal ribosome entry site (IRES) (from the
encephalomyocarditis virus), which in turn is followed by the gene encoding green
fluorescent protein (GFP). Transcription of integrated proviral sequences in infected
16 cells thus produces a bi-cistronic mRNA that encodes both NGN1 and GFP. Infected
cells can therefore be visualized by virtue of GFP fluorescence (Figure 2, lower,
arrows) or by immunostaining with anti-myc tagged GFP or anti-GFP.

Neural crest stem cells (NCSCs), cultured as previously described (Stemple *et al.* 1992.
21 Cell 71:973-985; Lo et al. 1998. Development 125:609-920) were fixed 2.5 days post
infection with NGN1-IRES-GFP retrovirus and analyzed at clonal density. The results
indicate that virtually all cells that are GFP+ (Fig. 4C, arrows) have a process-bearing,
neuronal morphology (Fig. 4A, arrows) and express neurofilament 160 Kd subunit
(NF160) (Fig. 4b, arrows). Such differentiation occurs rapidly and is detectable after 2-
26 2.5 days. Cells not expressing GFP have a flat morphology (Figure 4C, Figure 4A) and
do not express NF160 (Figure 4B). In high density cultures where neuronal
morphology is not easily distinguished (Figure 5A), NGN1-expressing cells (Figure
5C) can be seen to express NeuN (Figure 5B), a neuron-specific nuclear protein. No
such induction of neuronal markers is observed when cells are infected with a control
31 virus encoding GFP-IRES-Alkaline Phosphatase.

1 These data are similar to those obtained with a MASH1-IRES-GFP retrovirus (Fig. 3A-C), except that neuronal differentiation is much more efficient with the NGN1 retrovirus: essentially all NGN1-infected cells express a neuronal phenotype, while only 16% of MASH1-infected cells undergo neurogenesis under these conditions (Lo et al., 1998).

6 Forced expression of NGN1 thus promotes a core program of neurogenesis in neural crest stem cells. We predict that NGN1 will similarly promote neurogenesis in neural stem cells from the CNS. Thus, introduction of NGN1 coding sequences into undifferentiated neural crest stem cells can be used to efficiently promote neuronal differentiation of these cells, without the need to manipulate their cell culture environment.

Example 2

Expression of neuronal genes in non-neuronal cells by expression of NGN1

16 Murine NGN1 was expressed in cultured chick embryo fibroblasts (CEFs), using a replication-competent avian retroviral vector (RCAS). In this case, GFP was not used as a marker; rather a myc epitope tag was fused to the *ngn1* coding sequence to allow identification of infected cells by immunocytochemistry using a monoclonal antibody to the myc tag (9E 10). Cells were harvested have 5 days. Culture conditions are cited in Perez et al. (1999) Development 126:1715-1728.

21 Expression of NGN1 in CEFs caused induction of a number of markers of neuronal differentiation, including neuron-specific beta-tubulin (Figure 6D), neurofilament (NAPA-73; Figure 6B), and NF160 (Figure 6C). In addition the cells displayed morphological changes suggestive of neuronal differentiation. No induction of these markers was detected in control cultures infected with a retrovirus harboring luciferase gene (data not shown).

26 These data indicate that forced expression of NGN1 can elicit expression of at least some neuronal phenotypic markers even in non-neuronal cells. Thus, introduction of

1 NGN1 coding sequences into certain non-neuronal cell types, which may be more
easily accessed by biopsy than neural stem cells, may be used to promote expression of
some neuronal properties which may offer therapeutic benefit in an appropriate
transplantation setting. Such an approach would be particularly amenable to
autografting.

6 Example 3

Induction of tyrosine hydroxylase (TH) expression in NCSCs by forced expression of Phox2a

11 To induce TH expression, cultured rat NCSCs were infected with a retrovirus vector
expressing the paired homeodomain protein, Phox2a (Lo *et al.*, Development, 1998.
125:609-620). The Phox2a protein contained a myc epitope tag to permit visualization
of the expressed protein in infected cells by immunocytochemistry using an anti-myc
monoclonal antibody.

16 NCSCs were infected and the percentage of retrovirally-infected clones (clones
containing any myc-tag positive cells) containing at least one TH⁺ cell was determined
after 96 hours of growth a clonal density under the indicated conditions.
Approximately 10% of all infected clones contained detectable levels of TH (Figure 7),
21 and within these clones about 2.5% of all cells were TH-positive (Figure 8). No
induction of TH was seen using a control retrovirus encoding a myc-tagged form of
GFP (Figure 10F). Inclusion of forskolin in the culture medium (which increases
intracellular cAMP) increased the percentage of infected (myc-tag⁺) clones expressing
TH to about 50% (Figure 7), and within these clones almost 15% of the cells were TH⁺
26 (Figure 8). In contrast to the result obtained using NGN1, the TH⁺ cells produced by
forced expression of Phox2a did not have a neuronal morphology (Figure 9A) and did
not express pan-neuronal markers such as NF160 (not shown). The percentage of
infected cells expressing TH could be further increased to almost 35% (Figure 8), by
inclusion of additional factors such as GDNF and dexamethasone (DEX) together with
31 forskolin (Lo *et al.* (1999) Neuron. 22:693-705).

1 We predict that simultaneous expression of both Phox2a and NGN1 in the same cell
would cause the differentiation of neurons that express TH. Such neurons might be
useful for transplantation in Parkinson's Disease. The ability to uncouple the
expression of neuronal subtype properties from the expression of pan-neuronal
properties implies that it should be possible to control the differentiation of neural stem
6 cells to particular neuronal subtypes by expressing in them appropriate combination of
transcription factors.

Example 4

11 Induction of neuronal differentiation in neural crest stem cells by forced expression of NGN1 and Phox2a.

Neural crest stem cells (NCSCs) are co- infected with the NGN1-IRES-GFP and the
Phox2a retroviruses described above and grown at clonal density. Virtually all cells
that are GFP+ have process-bearing, neuronal morphology, express neurofilament 160
Kd subunit (NF160), NeuN, and TH. The differentiation occurs rapidly and is
16 detectable after 2-2.5 days. The cell is terminally differentiated and have membrane
conductance potential similar to catecholamine producing neurons of the PNS.

Example 5

21 Induction of Brn-3.0 in neural crest cells

Neural crest cells were infected with the retrovirus, NGN1-IRES-GFP, and grown in
mass culture. Following 3-5 days of culture, the cells developed a neuronal
morphology and expressed Brn-3.0, which is a sensory neuron-specific marker,
characteristic of dorsal root ganglion primary sensory neurons. (Greenwood and
Anderson. (1999). Development 126: 3543-3559)

1 What is claimed is:

1. A method of inducing a non-neuronal cell to differentiate into a neuronal cell, comprising:

6 contacting said non-neuronal cell with an expression vector comprising a neurogenin nucleic acid operatively linked to a promoter functional in said non-neuronal cell, wherein said neurogenin nucleic acid hybridizes under high stringency conditions to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11, or complements thereof, wherein said neurogenin nucleic acid is expressed in said cell.

11

2. The method according to Claim 1, wherein said non-neuronal cell is an embryonic stem cell.

16

3. The method according to Claim 1, wherein said non-neuronal cell is a neural stem cell.

4. The method according to Claim 3, wherein said neural stem cell is a neural crest stem cell.

21

5. The method according to Claim 1, wherein said non-neuronal cell is a fibroblast.

6. The method according to Claim 5, wherein said fibroblast is a chick embryo fibroblast.

26

7. The method according to Claim 1, wherein said nucleic acid encodes a neurogenin-1.

31

8. The method according to Claim 1, wherein said expression vector further comprises a sequence encoding a selectable marker.

1 9. The method according to Claim 8, wherein said selectable marker is a drug resistance marker.

 10. The method according to Claim 8, wherein said selectable marker is an epitope tag.

6 11. The method according to Claim 1, wherein said expression vector comprises a plasmid.

 12. The method according to Claim 1, wherein said vector comprises a retrovirus vector.

 13. The method according to Claim 1, wherein said vector comprises a herpesvirus vector.

16 14. A method of inducing a non-neuronal cell to express a core program of neurogenesis, comprising:

 contacting said non-neuronal cell with an expression vector comprising a neurogenin nucleic acid operatively linked to a promoter, wherein said neurogenin nucleic acid hybridizes under high stringency conditions to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11, or complements thereof; wherein said neurogenin nucleic acid is expressed in said non-neuronal cell.

 15. The method according to Claim 14, wherein said core program of neurogenesis comprises expression of beta-tubulin, neurofilament, NeuN and/or NF160.

 16. A method of inducing a non-neuronal cell to express a neuronal marker, comprising:

31 contacting said non-neuronal cell with an expression vector comprising a

1 Phox2a nucleic acid operatively linked to a promoter functional in said non-neuronal
cell, wherein said Phox2a nucleic acid hybridizes under high stringency conditions to
SEQ ID NO:13 or complements thereof, wherein said Phox2a nucleic acid is expressed
in said non-neuronal cell.

6 17. A method of inducing a non-neuronal cell to express a neuronal marker,
comprising:

contacting said non-neuronal cell with an expression vector comprising a
Phox2b nucleic acid operatively linked to a promoter functional in said non-neuronal
cell, wherein said Phox2b nucleic acid hybridizes under high stringency conditions to
11 SEQ ID NO:15 or complements thereof, wherein said Phox2b nucleic acid is expressed
in said non-neuronal cell.

18. The method according to Claim 16 or 17, wherein said neuronal marker is
an enzyme produced by neurons that synthesize a catecholamine neurotransmitter.

16

19. A method of inducing a non-neuronal cell to differentiate into a neuron,
comprising:

contacting said cell with a neurogenin nucleic acid operatively linked to a first
promoter and with a Phox2a nucleic acid operatively linked to a second promoter,
21 wherein said first and second promoters are functional in said non-neuronal cell and
said neurogenin nucleic acid hybridizes under high stringency conditions to SEQ ID
NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11,
or complements thereof and said Phox2a nucleic acid hybridizes under high stringency
conditions to SEQ ID NO:13 or its complement, wherein said neurogenin and said
26 Phox2a nucleic acids are expressed in said non-neuronal cell.

20. The method according to claim 18, wherein said neuron is a catecholamine-
synthesizing neuron.

31 21. A non-neuronal cell having an neuronal phenotype induced by an

1 expression vector comprising a neurogenin nucleic acid operatively linked to a first
promoter and a Phox2a nucleic acid operatively linked to a second promoter, wherein
said first and second promoters are functional in said cell and said neurogenin nucleic
acid hybridizes under high stringency conditions to SEQ ID NO:1, SEQ ID NO:3, SEQ
ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11, or complements thereof
6 and said Phox2a nucleic acid hybridizes under high stringency conditions to SEQ ID
NO:13 or its complement.

22. A method of identifying an agent that modulates neurogenesis in a
transformed non-neuronal cell comprising an expression vector comprising a
11 neurogenin nucleic acid operatively linked to a promoter functional in said transformed
cell, wherein said neurogenin nucleic acid hybridizes under high stringency conditions
to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ
ID NO:11, or complements thereof comprising the steps of:
a) contacting said transformed cell with said agent; and
16 b) detecting a modulation in the induction of neurogenesis in said transformed
cell.

23. A method of identifying an agent that modulates the induction of a neuronal
subtype-specific phenotype in a transformed non-neuronal cell comprising an
21 expression vector comprising a Phox2a nucleic acid operatively linked to a promoter
functional in said transformed cell, wherein said Phox2a nucleic acid hybridizes under
high stringency conditions to SEQ ID NO:13 or complements thereof comprising the
steps of:
a) contacting said transformed cell with said agent; and
26 b) detecting a modulation in the induction of a neuronal subtype-specific
phenotype in said transformed cell.

24. A method of identifying an agent that modulates the induction of a neuronal
subtype-specific phenotype in a transformed non-neuronal cell comprising an
31 expression vector comprising a Phox2b nucleic acid operatively linked to a promoter

- 1 functional in said transformed cell, wherein said Phox2b nucleic acid hybridizes under
high stringency conditions to SEQ ID NO:15 or complements thereof comprising the
steps of:
- a) contacting said transformed cell with said agent; and
 - b) detecting a modulation in the induction of a neuronal subtype-specific
- 6 phenotype in said transformed cell.

1 / 29

(1). Rat Neurogenin cDNA Sequence

1 ATCCGGAGCT GATCTGATCG CCGGCGACAT CAGTCGGGAG ACCAGCCCGG
51 CGCGTGGCCC CCTGCAGGCG AGGCGAGGAG GCCAAGCCCA TTCCCTCCCT
101 GAGCCCCCTGC GATCTTCCCC GGCCCTCGCG CCTGCAGCAG GCACAGGCTA
151 GCCCCGGGTC ATACGGACAG TAAGTGCGCT TCGAAGGCCG TGCACTCGGC
201 CCACATTCAA GCCCTCCAAA CCTCCCGTCC GTCCGTCCGT CCTGCAACGA
251 TGCCCTGCCCC TTTGGAGACC TGTCTCTCTG ACCTCGACTG CGCCAGCAGC
301 AACAGCGGGA GCGACCTGTC CAGTTTCCTC ACCGACGAGG AGGACTGTGC
351 CAGGCTCCAG CCCCTAGCTT CCACCTCAGG GCTGTCCGTG CCAGCCCCGA
401 GGAGCGCGCC CACCCTCTCC GGGGCATCGA ACGTTCCCGG TGGCCAGGAC
451 GAAGAGCAGG AGCGGCGCG ACGGCGAGGT CGCGCGCGGG TGCGGTCCGA
501 GCGGCTGCTG CACTCGCTGC GGAGGAGCCG TCGCGTCAAG GCCAACGATC
551 GCGAGCGCAA CCGTATGCAT AACCTCAACG CTGCGCTGGA CGCTCTGCGC
601 AGCGTGCTGC CCTCGTTCCC CGACGACACC AAGCTCACCA AGATTGAGAC
651 GCTGCGCTTC GCCTACAACT ACATCTGGGC CCTGGCTGAG ACACTGCGCC
701 TGGCAGATCA AGGGCTCCCC GGGGCGGTG CCGGGAGCG CCTCCTGCCT

FIG. 1A-1

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751 CCGCAGTGTG TCCCCCTGCCT GCCCGGTCCC CCGAGCCCGG CCAGCGATAC
801 AGAGTCCTGG GGCTCCGGGG CCGCTGCCTC CCCCCTGCGCT ACTGTGGCGT
851 CACCACTCTC TGACCCCAGT AGTCCCCTCGG CTTCAGAAGA CTTACCTAT
901 GGCCCCGGTG GTCCCCCTTT CTCCTTTCCT GGCTGCCCCA AAGACCTCCT
951 CCATACGACA CCGTGCTTCA TCCCGTACCA CTAGGGCTTT GCAAGACAAC
1001 GTTAATACTT CTTTCCTGCC CAGTCTATG AGCAATAGAT GGGGAGCCG
1051 GCTGAAGCCT CGGGGAGCAC CCTTACCCCC AGGTGGATGC TGGGAGCTTT
1101 AAAGAGGGA GGGATACCTG ACCACTTGCT AGGTGCCGC ACCCTCGCTG
1151 AGAAGCTGCC CCTCGGACTG TTTCCCCACG CCCCAGCACC GGGCCCCCTCC
1201 TGCCCCGCC CCAGACGGGC TTTTCGGTTTT TTTTITGGAC TTCTTGAAT
1251 TCACAAAACC TCCTTTGTGA CTGGCTCAGA ACTGACCCCA GCCACCACTT
1301 CAGTGTGATT TGGAAAAGG ACAGATGAGC CCTGAAGAC GAGGTGAAA
1351 GTCAATTTTA CAATTGTAG AACTCTAATG AAGAAAAACG AGCATGAAA
1401 TTCGGTTTGA GCCGGCTGAC AATACAATGA AAAGGCTTAA AAAAAAGGAG
1451 ACACAAGGAG TGGGCTTCAT GCATTATGGA TCCCGACCCC CACCCTGTG
1501 GGCTTGCTCC CGGAAGAACT GAGTGCT

FIG. 1A-2

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Met Pro Ala Pro Leu Glu Thr Cys Leu Ser Asp Leu Asp Cys Ala Ser
 1 5 10 15
 Ser Asn Ser Gly Ser Asp Leu Ser Ser Phe Leu Thr Asp Glu Glu Asp
 20 25 30
 Cys Ala Arg Leu Gln Pro Leu Ala Ser Thr Ser Gly Leu Ser Val Pro
 35 40 45
 Ala Arg Arg Ser Ala Pro Thr Leu Ser Gly Ala Ser Asn Val Pro Gly
 50 55 60
 Gly Gln Asp Glu Glu Gln Glu Arg Arg Arg Arg Arg Gly Arg Ala Arg
 65 70 75 80
 Val Arg Ser Glu Ala Leu Leu His Ser Leu Arg Arg Ser Arg Arg Val
 85 90 95
 Lys Ala Asn Asp Arg Glu Arg Asn Arg Met His Asn Leu Asn Ala Ala
 100 105 110
 Leu Asp Ala Leu Arg Ser Val Leu Pro Ser Phe Pro Asp Asp Thr Lys
 115 120 125
 Leu Thr Lys Ile Glu Thr Leu Arg Phe Ala Tyr Asn Tyr Ile Trp Ala
 130 135 140
 Leu Ala Glu Thr Leu Arg Leu Ala Asp Gln Gly Leu Pro Gly Gly Gly
 145 150 155 160
 Ala Arg Glu Arg Leu Leu Pro Pro Gln Cys Val Pro Cys Leu Pro Gly
 165 170 175
 Pro Pro Ser Pro Ala Ser Asp Thr Glu Ser Trp Gly Ser Gly Ala Ala
 180 185 190
 Ala Ser Pro Cys Ala Thr Val Ala Ser Pro Leu Ser Asp Pro Ser Ser
 195 200 205
 Pro Ser Ala Ser Glu Asp Phe Thr Tyr Gly Pro Gly Gly Pro Leu Phe
 210 215 220
 Ser Phe Pro Gly Leu Pro Lys Asp Leu Leu His Thr Thr Pro Cys Phe
 225 230 235 240
 Ile Pro Tyr His

FIG. 1B

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(2). Mouse Neurogenin cDNA Sequence (Open Reading Frame)

```
1  ATGCCTCCCC CTTTGGAGAC CTGCATCTCT GATCTCGACT
41  GCTCCAGCAG CAACAGCAGC AGCGACCTGT CCAGCTTCCT CACCGACGAG
91  GAGGACTGTG CCAGGGCTACA GCCCCCTAGCC TCCACCTCGG GGCTGTCCGT
141 GCCAGCCCCG AGGAGCGCTC CCGCCCTCTC CGGGGCATCG AATGTTCCCG
191 GTGCCCCAGGA CGAAGAGCAG GAACGGCGGA GCGGGCGAGG TCGGCTCGG
241 GTGCGGTCCG AGGCTCTGCT GCACTCCCTG CGGAGGAGTC GTCGCGTCAA
291 AGCCAACGAT CGCGAGCGCA ACCGCATGCA CAACCTCAAC GCTGCGCTGG
341 ACGCCTTGCG CAGCGTGCTG CCCTCGTTCC CCGACGACAC CAAGCTCACC
391 AAGATTGAGA CGCTGCGCTT CGCCTACAAC TACATCTGGG CCCTGGCTGA
441 GACACTGCGC CTGGCAGATC AAGGGCTCCC CGGGGGCAGT GCCCGGGAGC
491 GCCTCCTGCC TCCGCAGTGT GTCCCCCTGTC TGCCCCGGGCC CCCGAGCCCCG
541 GCCAGCGACA CTGAGTCCTG GGGTTCCGGG GCCGCTGCCT CCCCCTGCGC
591 CACTGTGGCA TCACCACTCT CTGACCCCCAG TAGTCCCTCG GCTTCAGAAG
641 ACTTCACCTA TGGCCCCGGC GATCCCCCTT TCTCCTTTCC TGGCCTGCCC
691 AAAGACCTGC TCCACACGAC GCCCTGTTTC ATCCCATACC ACTAGTAA
```

FIG. 1C

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Met Pro Pro Pro Leu Glu Thr Cys Ile Ser Asp Leu Asp Cys Ser Ser
 1 5 10 15
 Ser Asn Ser Ser Ser Asp Leu Ser Ser Phe Leu Thr Asp Glu Glu Asp
 20 25 30
 Cys Ala Arg Leu Gln Pro Leu Ala Ser Thr Ser Gly Leu Ser Val Pro
 35 40 45
 Ala Arg Arg Ser Ala Pro Ala Leu Ser Gly Ala Ser Asn Val Pro Gly
 50 55 60
 Ala Gln Asp Glu Glu Gln Glu Arg Arg Arg Arg Arg Gly Arg Ala Arg
 65 70 75 80
 Val Arg Ser Glu Ala Leu Leu His Ser Leu Arg Arg Ser Arg Arg Val
 85 90 95
 Lys Ala Asn Asp Arg Glu Arg Asn Arg Met His Asn Leu Asn Ala Ala
 100 105 110
 Leu Asp Ala Leu Arg Ser Val Leu Pro Ser Phe Pro Asp Asp Thr Lys
 115 120 125
 Leu Thr Lys Ile Glu Thr Leu Arg Phe Ala Tyr Asn Tyr Ile Trp Ala
 130 135 140
 Leu Ala Glu Thr Leu Arg Leu Ala Asp Gln Gly Leu Pro Gly Gly Ser
 145 150 155 160
 Ala Arg Glu Arg Leu Leu Pro Pro Gln Cys Val Pro Cys Leu Pro Gly
 165 170 175
 Pro Pro Ser Pro Ala Ser Asp Thr Glu Ser Trp Gly Ser Gly Ala Ala
 180 185 190
 Ala Ser Pro Cys Ala Thr Val Ala Ser Pro Leu Ser Asp Pro Ser Ser
 195 200 205
 Pro Ser Ala Ser Glu Asp Phe Thr Tyr Gly Pro Gly Asp Pro Leu Phe
 210 215 220
 Ser Phe Pro Gly Leu Pro Lys Asp Leu Leu His Thr Thr Pro Cys Phe
 225 230 235 240
 Ile Pro Tyr His

FIG. 1D

SUBSTITUTE SHEET (RULE 26)

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(3). X-ngnr-1a cDNA Sequence:

```
1  GCGTGTCA CA CGGCAGTTGC ACTCATAATA CACTGTGAGC TGACAGTCGC
51  AACCAAGCCC GACAGGGAAC ACGCAGCAAG TCTACTGCAC GACTATAACC
101 CGACGACTCG ACCCAACTCA CCTGCTGCTT CAGGGGCCAA ACACCAAGTT
151 ATAAAGTAAG TAACTTCCAT TGCAACTGCA GCATTGTCAC TTGCGACAGC
201 GCATGAAGTA GTGAGAGGCA CAGACCATGT ACATATATGG GGTGTGTGGT
251 TATTATAGTA AGTGGGATGA TGTTTGGGTT ATTATAGTAA GTGGATGTGA
301 AGTTGTCA GT GCAACATTGG GGCTAACCAT TGGCTGTGTG TTTGCCGCTTG
351 TCTAGGATGG TGCTGCTCAA GTGCGAGTAC CGCGATGAAG AGGAGGACCT
401 GACCTCTGCC TCCCCCTGCT CCGTGACCTC CTCTTTCCGT TCCCCGGCGA
451 CGCAGACGTG CAGCTCGGAC GATGAGCAGC TCCTGAGTCC CACCAGCCCG
501 GGACAGCACC AGGGGAAGA GAACAGCCCG CGATGCAGGA GGAGCCGAGG
551 CCGCGCTCAG GGCAAGAGCG GAGAACTGT GTTAAAGATC AAGAAGACCC
601 GCGCGGTTAA AGCTAACAA CCGGAAAGGA ATCGCATGCA CAACCTGAAC
651 TCTGCGCTTG ATTCCCTCAG GGAAGTGTG CCCTCTTTAC CTGAAGATGC
701 CAAACTCACC AAGATAGAGA CCTGCGCTT TGCCTACAAC TACATCTGGG
```

FIG. 1E-1

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751 CTCTTAGCGA AACTTTGCGC CTTGGCGACC CAGTGCACCG ATCTGCTTCC
801 ACCCCAGCAG CAGCCATATT GGTGCAGGAC TCCTCTTTCAT CCCAGAGCCC
851 CTCCCTGGAGC TGCAGCTCGT CCCCCTTCTTC CTCCTTGTTGC TCCTTCTCCC
901 CGGCCAGCCC TGCCAGCTCC ACCTCGGACA GTATTGAGTC CTGGCAGCCC
951 TCTGAGCTCC ACCTGAACCC CTTTCATGTCT GCCAGCAGCG CTTTCATTG
1001 AACTCCCTGTT GGACTATGAT GGATTCCTCAC ACTTCCAATT GCTACATATG
1051 AAGAAATACCT CAGTGGGGCC CCAGTGCAAA TGATTTTCCT GGGAAACCCAG
1101 TTTATTGAGC ATGAGCCCCAT ATAGTGTAAT AATATCATCC TGCAGTGACC
1151 AAATTGCACT CTGTGGGTTC TGCTGATGGG GAGAAAGTGGG GGGCTTGATC
1201 CCCCTGAGTT TGTGCTTACC TGTATAGCAT TTAATCCCCC TGCTGTCATG
1251 CCCCTGGCAT ATGATGGAGT ACATTGCTGG GTCTATTTTA TTATCAGCAA
1301 TGTGAACTGA AA

FIG. 1E-2

SUBSTITUTE SHEET (RULE 26)

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Met	Val	Leu	Leu	Lys	Cys	Glu	Tyr	Arg	Asp	Glu	Glu	Glu	Asp	Leu	Thr	1	5	10	15
Ser	Ala	Ser	Pro	Cys	Ser	Val	Thr	Ser	Ser	Phe	Arg	Ser	Pro	Ala	Thr	20	25	30	
Gln	Thr	Cys	Ser	Ser	Asp	Asp	Glu	Gln	Leu	Leu	Ser	Pro	Thr	Ser	Pro	35	40	45	
Gly	Gln	His	Gln	Gly	Glu	Glu	Asn	Ser	Pro	Arg	Cys	Arg	Arg	Ser	Arg	50	55	60	
Gly	Arg	Ala	Gln	Gly	Lys	Ser	Gly	Glu	Thr	Val	Leu	Lys	Ile	Lys	Lys	65	70	75	80
Thr	Arg	Arg	Val	Lys	Ala	Asn	Asn	Arg	Glu	Arg	Asn	Arg	Met	His	Asn	85	90	95	
Leu	Asn	Ser	Ala	Leu	Asp	Ser	Leu	Arg	Glu	Val	Leu	Pro	Ser	Leu	Pro	100	105	110	
Glu	Asp	Ala	Lys	Leu	Thr	Lys	Ile	Glu	Thr	Leu	Arg	Phe	Ala	Tyr	Asn	115	120	125	
Tyr	Ile	Trp	Ala	Leu	Ser	Glu	Thr	Leu	Arg	Leu	Gly	Asp	Pro	Val	His	130	135	140	
Arg	Ser	Ala	Ser	Thr	Pro	Ala	Ala	Ala	Ile	Leu	Val	Gln	Asp	Ser	Ser	145	150	155	160
Ser	Ser	Gln	Ser	Pro	Ser	Trp	Ser	Cys	Ser	Ser	Ser	Pro	Ser	Ser	Ser	165	170	175	
Cys	Cys	Ser	Phe	Ser	Pro	Ala	Ser	Pro	Ala	Ser	Ser	Thr	Ser	Asp	Ser	180	185	190	
Ile	Glu	Ser	Trp	Gln	Pro	Ser	Glu	Leu	His	Leu	Asn	Pro	Phe	Met	Ser	195	200	205	
Ala	Ser	Ser	Ala	Phe	Ile											210			

FIG. 1F

SUBSTITUTE SHEET (RULE 26)

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X-ngnr-1b cDNA Sequence:

```
1  CGAGTGCGCA ACACTTGAGC TGGAGTGCGG GGCGCGTGTC ACACACACAC
51  TGAAGTGCCA CTGACACCAG AGACACAGCG AGTGGGAACC CCCTGCTACT
101 ACAGGACTAG GAGAAAAGCC GCACAGCCTG CAGCGCCGCA ACCCGACTCA
151 CCTGCTGCTC CCGGAGCCAC AAGCCTGGCG CACAAGATGG TGCTGCTGAA
201 GTGCGAATAC CGCGATGAGG TGTGCGAACT GACCTCTGTC TCCCCCTGCT
251 CCGTGTCCCTC CTCCTCTTCA CACCCGTCCC CGGCGATGCA GACGTGCAGC
301 TCGGACGATG AGCAGCTACA CAGTCCGACA AGCCCCGACGC TCACGCACCT
351 GCAGCAGGGA CGGACCAGG GGGAGGAGAA CAGCCCCGCGA TGCAGGAGGA
401 GCCGAGCCCG CGGAGACACC GTGCTGAAGA TCAAGAAAGAC CCGGCGCGTT
451 AAAGCCAATA ACCGCGAGAG GAATCGCATG CACCACCTGA ACTATGCGCT
501 CGATTCTCTG AGGAGGTTTC TACCGTCATT ACCCGAAGAC GCCAAACTCA
551 CCAAGATAGA GACCTTGCGC TTTGCCCCACA ACTACATCTG GGCTCTTAGC
601 GAAACTTTGC GCCTGGCCGA CCAGCTGCAC GGATCTACTT CCACCCCAGC
651 AGCAGCCATA TTGGTACAGG ACTCCTATCC TTCCCCTGAGC CCTTCCCTGGA
701 GCTGCAGCTC GTCCCCATCC TCCAACCTCTT GCGACTCCTT CTCCCCGACC
```

FIG.-1G-1

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751 AGCCCTGCCA GCTCCACCTC GGACAGTATT GAGTACTGGC AGCCCTCTGA
801 GCTCCGCTTG AACCCCTTCA TGCTGCCCCT TTGAACGCAC AGGACTATGG
851 GTGATTTTAA CTTTTTACAC TTTAAATTCC TGCTTCCCAT AAGGGTCAAG
901 TACTGCAGGG GTTACATATC AAGTTTACCT CAGGGGGGGC CACAGCAAAT
951 TCTTTTCCCTG GGCCCTAAAA TGTCCTCTGA ATTTGAGCCC ATATAGTGA
1001 ATGGTATAAC CCTGCAATGG TATAATCCAG CAATGGTATA ATCCTGCATC
1051 GTTACCCTAAT TGTACTTTGT GGGGTCTGCT GATGGGGGAC AAGTGTTTGA
1101 CCTGTGTCCA GAGTTTCACA TTTACTCCCC CTTTGGTAT ATCTCTGGCC
1151 GCAACACTTG CTGTGTCTGT TTCATCGTTA GCTATGTGTA TTAGGAAACT
1201 GTCTATCCCCT CATCTGCACC TGTTAGACTA CAGCTACCAA CTTCTGTGTA
1251 CCAGGGGGCT ACTGGGTAAT GTACTTC

FIG. 1G-2

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MVLLKCEYRDEVSELTSPSCSVSSSSSHPSAMQTCSSDDEQL
 HSPTSPTLTHLQQGRDQGEENSPRCRRSRARGDTVLKIKKTRRVKANNRERNRMHHLN
 YALDSLREVLPSLPEDAKLTKIETLRFAHNYIWAALSETLRLADQLHGSTSTPAAAILV
 QDSYPSLSPSWSCSSPSSNSCDSFSPTSPASSTSDSIEYWQPSLRLNPFMSAL

FIG._1H

MDYSYLNsyDSCVAAMEASAYGDFGACSQPGGFQYSPLRPAFAAGPPCPALGSSNCALGALRDHQP
 APYSAPVYKFFPEPSGLHEKRKQRRIRTTFTSAQLKELELVFAETHYPDIYTREELALKIDLTEARV
 QVWFQNRRAKFRKQERAAAKGAGATGAKKGEARCSSEDDDSKESTCSTPDSTASLPPPPAPSLA
 SPRLSPSPLPAALGSGPGPPQLKGALWAGVAGGGGGPGTGAAELLKAWQPAEPGPGPFSGVLSSFH
 RKPGPALKTNLF

FIG._2B

MYKMEYSYLNSSAYESCMAGMTSSLASAYADFSSCSQASGFQYNPIRTTFGATSGCPSLTPGSCSLGTLR
 DHQSSPYAAVPYKLFTHGGLNEKRKQRRIRTTFTSAQLKELELVFAETHYPDIYTREELALKIDLTEARV
 QVWFQNRRAKFRKQERAAAAAANKGSSGKKSDSRDDDESKEAKSTDPDSTGGPGPNPNTPSCGANGG
 GGGPSPAGAPGAAGPGPGGEPGKGGAAAAAAGGLAAAGGPGQGWAPGPGPITSIP
 DSLGGPFASVLSLQRPNGAKAALVKSSMF"

FIG._2D

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Available Sequence of 30R (Restriction Fragment of Neurogenin2 (ngn2) Genomic Clone);
Includes Full Length Open Reading Frame of ngn2

```
1  CTTAGGAAGCGCCCAAGCCCGGAGCGGAGGACACCGTGCTCGGTTCCGGGTGGGGGACA 60
   -----+-----+-----+-----+-----+-----+
   GAATCCTTCGCGGTTTCGGGGCCTCGCCCTCCTGTGGCACGAGCCAAGGCCACCCCTGT
61  TTCCCGGACACACCGGAGCAGCAGCTGCGCCGGAAACATTGGAGCCGCGTAGGTAAGTG 120
   -----+-----+-----+-----+-----+-----+
   AAGGCCCTGTGTGGCCTCGTCTGTCGACGGGCCTTGTAACCTCGGCGCATCCATTAC
121 TGCAATGCCCGGGCTTTCCATTTCGCAGGCAGTGTCCCCACGCAGGCTCACGCCGCCACGC 180
   -----+-----+-----+-----+-----+-----+
   ACGTACGGCGCCGAAAGGTAAGCTCCGTCACAGGGTGCGTCCGAGTGCGGCGGGTGCG
181 TAACTCCATCGTTTAGACGCAGTGACTTCTGTGACCGGCAGAGGTGGCTCGAGCCCCGGG 240
   -----+-----+-----+-----+-----+-----+
   ATTGAGGTAGCAAAATCTGCGTCACTGAAGACACTGGCCGCTCTCCACCGAGCTCGGGCCC
241 GCGCTCCTCCCCAGCTCTGTCTCTCGCCATCTTCGGGAATGCACATTGAGGGAGATGGAGG 300
   -----+-----+-----+-----+-----+-----+
   CGCGAGGAGGGTTCGAGACAGGAGCGGTAGAAGCGCTTACGTGTAACCTCCCTACCTCC
301 GGGGGGGCGGGCGGCCAGCGACACTTACCCCTGTCCATTCTGGGAATAAATTTC 360
   -----+-----+-----+-----+-----+-----+
   CCCCCCGCCCGCCGGTTCGCTGTGAATGGGACAGGTAAGACCCTTATTAAAG
```

FIG. 11-1

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ATCTGCCTCTTCTTCTCAGGATGTTCTGTCATAATCTGAGACTCTGGAGTTGAAGAGGAA 420
 -----+-----+-----+-----+-----+-----+-----+
 TAGACGGAGAAGAGTCTCTACAGCAGTTTAGACTCTGAGACCTCAACTTCCTCCTT
 361
 M F V K S E T L E L K E E -
 GAGGAGTACTGATGCTGCTGGGCTCGGCTTCCCGGCCCTCGGCGACCCCTGACCCCGATG 480
 -----+-----+-----+-----+-----+-----+-----+
 CTCCATGACTACGACGACCCGAGCCGAAGGGCCGGAGCCGCTGGGACTGGGGCTAC
 421
 E E V L M L L G S A S P A S A T L T P M -
 TCCTCCAGCGGACGAGGAGGAGGAGGAGTGCGCCGGCGGCTCCGCGGTGGG 540
 -----+-----+-----+-----+-----+-----+-----+
 AGGAGTGGCGCCTGCTCCTCCTGCTCCTCGACGGCGCGGCCGAGGCGCGCACCC
 481
 S S A D E E D E E L R R P G S A R G -
 CAGCGTGGAGCGGAAGCCGAGCAGGGGTGCAGGGCAGTCCGGCGTCCGGTCCGGGGT 600
 -----+-----+-----+-----+-----+-----+-----+
 GTCGACCTCGCCTCGGCTCGTCCCCACGTCCCGTCAGGCCGACGCCACGGCCCCCA
 541
 Q R G A E A E Q G V Q G S P A S G A G G -
 TGCCGGCCAGGGCGGTGCTGGGCTGATGCACGAGTGCAAGCGTCGCCCGTCCGCTCA 660
 -----+-----+-----+-----+-----+-----+-----+
 ACGGCCGTCCCGCGACGACCCGGACTACGTGCTCACGTTCCGACGGGCGAGCGCGAGT

FIG. 11-2

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a C R P G R L L G L M H E C K R R P S R S -
CGGGCCGTCTCCCGAGGTGCCAAGACGGCGGAGACGGTGACGCGCATCAAGAAGACCCGC
661 -----+-----+-----+-----+-----+ 720
GCCCGGAGAGGGCTCCACGGTTCTGCCGCCCTCTGCCACGTCGCGTAGTTCTTCTGGGGC

a R A V S R G A K T A E T V Q R I K K T R -
AGGCTCAAGGCCAACACCGCGAGCGCAACCGCATGCACAACTAAACGCCGCGCTGGAC
721 -----+-----+-----+-----+-----+ 780
TCCGAGTTCGGGTGTGGCGCTCGCGTTGGCGTACGTGTGGATTTCGGCGCGGACCTG

a R L K A N N R E R N R M H N L N A A L D -
GCGCTGCGGAGGTGCTGCCACCTTCCCCGAGGATGCCAAGCTCACGAAGATCGAGACG
781 -----+-----+-----+-----+-----+ 840
CGCGACGCGCTCCACGACGGGTGGAAGGGCTCCTACGGTTCGAGTGCTTCTAGCTCTGC

a A L R E V L P T F P E D A K L T K I E T -
CTGCGCTTCGCCCCACAATTACATCTGGGCGCTCACCGAGACTCTGCGCCTGGCGGACCAC
841 -----+-----+-----+-----+-----+ 900
GACGCGAAGCGGGTGTAAATGTAGACCCCGGAGTGCGTCTGAGACGCGGACCGCCTGGTG

a L R F A H N Y I W A L T E T L R L A D H -
TGCGCCGCGCGGTGGCCTCCAGGGGCGCTCTTCACGGAGGCGGTGCTCCTGAGCCCCG
901 -----+-----+-----+-----+-----+ 960
ACGCGCGCGCGCACCGGAGGTCCCCCGGAGAGTGCCTCCGCCACGAGGACTCGGGC

FIG. 11-3

```

a      C A G A G G L Q G A L F T E A V L L S P -
      GGAGCTGCGCTCGGGCCAGCGGGACAGCCCTTCTCCACCTTCTCTCCTGGAGCTGCACC 1020
      -----+-----+-----+-----+-----+
      CCTCGACGCGAGCCGGTCCGCCCTGTCTGGGAAGAGGTGGAAGGAGGACCTCGACGTGG
      961

a      G A A L G A S G D S P S P S S W S C T -
      AACAGCCCGGCGTCACTCCAACTCCACGTCCCCATACAGCTGCACTTTATCGCCCGCT 1080
      -----+-----+-----+-----+-----+
      TTGTCTGGGCGCAGTAGGAGGTGAGGTGCAGGGGTATGTCGACGTGAATAGCGGGCGA
      1021

a      N S P A S S S N S T S P Y S C T L S P A -
      AGCCCCGGGTCAGACGTGACTACTGGCAGCCCCCACCTCCGGAGAAGCATCGTTATGCG 1140
      -----+-----+-----+-----+-----+
      TCGGGGCCAGTCTGCACCTGATGACCGTCTGGGGGTGGAGGCCCTCTCGTAGCAATACGC
      1081

a      S P G S D V D Y W Q P P P E K H R Y A -
      CCTCACCTGCCCCCTCGCCAGGACTGTATCTAGAGCTGCGGGTCTCCCTCTCTCGTCTCC 1200
      -----+-----+-----+-----+-----+
      1141

```

FIG. 11-4

```

a      P H L P L A R D C I *
      GGAGTGGACGGGAGCGGTCCCTGACATAGATCTCGACGCCCAGAGGGAGAGAGCAGAGG
      TACCCGGGCCCCCTCCTTCCCATCCTTCTCCCGCCCCCACCCCTCCACGCCCCGGAATCCAC
      1201 -----+-----+-----+-----+-----+
      ATGGCCCCGGAGGAGGGTAGGAAGAGGGGGGGGGTGGGAGGTGCGGGGCCCTTAGGTG
      TTCACAGAACAGAAGTTGGCCCCCTTGCAATCCCCCTCCGCGGCTGGTGCTTCGGGGTTGG
      1261 -----+-----+-----+-----+-----+
      AAGTGTCTTGCTTCAACCGGGAACGTTAGGGGAGGCGCCGACCAAGCCCCCAACC
      AAAACAACCTCTGTTTATTGAAATTAAGATTTTGTCAAAAAGAAATATGCTTTTGGAAAT
      1321 -----+-----+-----+-----+-----+
      TTTTGTGAGACCAATAACTTTAATTCTAAACCAGTTTTTCTTATACGAAACCTTA
      TGGGG
      1381 ----- 1385
      ACCCC

```

FIG. 11-5

Available Sequence of 17RB And 17B (Restriction Fragments of Neurogenin3 (ngn3) Genomic Clone;
Includes Full Length Open Reading Frame of ngn3

```

1  ATCTTTTGTAGTCGGGAGAACTAGGTAACAATTCGGAAACTCCAAAGGTGGATGAGGGG  60
   -----+-----+-----+-----+-----+-----+-----+
   TAAGAAAACTCAGCCCTCTTGATCCATTGTTAAGCCTTTGAGGTTTCCCACCTACTCCCC
61  CGCGCGGGGTGTGTGGGGATACTCTGTGTCCTCCCGTCGAGTGACCTCTAAGTCAGAGG  120
   -----+-----+-----+-----+-----+-----+-----+
   GCGGCCCCACACACACCCCTATGAGACCAGGGGCGACGTCACCTGGAGATTCACTCTCC
121 CTGGCACACACACACCTTCCATTTTTCCTCCACCGCAGGATGGCGCTCATCCCTTGGAT  180
   -----+-----+-----+-----+-----+-----+-----+
   GACCGTGTGTGGAAGGTAAATAAGGTTGGCGTCTACCGCGGAGTAGGGAACCTA
   M A P H P L D -
181 GCGCTCACCATCCAAGTGTCCCCAGAGACACAACAACCTTTTCCCGAGCCTCGGACCAC  240
   -----+-----+-----+-----+-----+-----+-----+
   CCGGAGTGGTAGGTTACAGGGGTCTCTGTGTTGTTGGAAAGGGCCTCGGAGCCTGGTG
   A L T I Q V S P E T Q Q P F P G A S D H -
241 GAAGTGCTCAGTTCCAATTCCACCCCCACCTAGCCCCACTCTCATACCTAGGACTGCTCC  300
   -----+-----+-----+-----+-----+-----+-----+
   CTTACGAGTCAAGGTTAAGTGGGGTGGATCGGGTGAGAGTATGGATCCCCTGACGAGG
   E V L S S N S T P P S P T L I P R D C S -
a
a

```

FIG. 1J-1

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GAAGCAGAAGTGGGTACTGCCGAGGAGACCTCGAGGAAGCTCCGGCGCCCGACGCGGAGGG
-----+-----+-----+-----+-----+ 360
CTTCGTCTTCACCCACTGACGGCTCCCTGGAGCTCCTTCGAGGCGGGGCTGCGCCTCCC
E A E V G D C R G T S R K L R A R R G G -
CGCAACAGGCCCCAAGAGCGAGTTGGCACTCAGCAACAGGAAGCCGCGCAAGAAG
-----+-----+-----+-----+ 420
GCGTTGTCCGGGTTCTCGCTCAACCGTGAGTCGTTGTGCTTCTTCGGCGCGTTCTTC
R N R P K S E L A L S K Q R R S R R K K -
GCCAATGATCGGAGOGCAATCGCATGCACAACTCAACTCGGGCGCTGGATGCGCTGCGC
-----+-----+-----+-----+ 480
CGGTACTAGCCCTCGGTTAGCGTACGTGTTGGAGTTGAGCCGCGACCTACGCGACGCG
A N D R E R N R M H N L N S A L D A L R -
GGTGTCCTGCCACCTTCCCGGATGACGCCAACTTACAAGATCGAGACCTCGCGCTTC
-----+-----+-----+-----+ 540
CCACAGGACGGGTGAAGGGCCTACTGCGGTTTGAATGTTTCTAGCTCTGGGACGCGAAG

FIG._1J-2

a L V F S D F L * -

FIG. 1J-3

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```

1  agctcgaatt ccgttgctgt cgggccgatg gactactcct acctcaattc gtacgattcg
61  tgcgtggcgg ccatggaggc gtccgcctac ggtgacttcg gcgcctgcag ccagcctgga
121  ggcttccaat acagtccctt gcggcctgcc ttccccgccg ctggggccacc ttgccccgcg
181  ctcggtcctt ccaactgtgc gcttggcgcc ctacgcgacc accagccccg accctactcg
241  gcagttccct acaagtcttt ccggagccg tccggcctgc atgagaagcg caagcagcgg
301  cgcatccgca caacgttcac gagtgtctag ctcaaggagt tggagcgctt ctctgcgag
361  accactacc ccgacattta cactcgcgag gaactggcgc tcaagatcga cctcactgag
421  gctcgcgtgc aggtcttggt ccagaaccgc cgggccaaagt tccgcaaaca ggagcgcgcg
481  gccagcgcca aaggcgcgcc ggagcgacg ggcccaaaa agggcgaggc gcgttgctcg
541  tcggaggacg acgactccaa ggagtccacg tgcagcccca cgcgcgacag caccgcgtcg
601  ctgccgcgcg cgctgcacc cagcctggcc agcccgctc tgagccccag ccctctgccc
661  gccgcgctgg gctccgggcc cgggccccag ccgctcaagg gagcgttgtg ggacgggtg
721  gcgggcggtg gaggtggcgg ccccggcacg ggcgacgagg agctgcttaa ggctggcag
781  ccggcggaac ccggggccagg tccctctctt ggagttctgt cctccttca ccggaagccc
841  ggccccgccc tgaagacaaa cctctcttag ccgcggcgtt ctgtaggcaa ccagcctgcc
901  ccgagagaga caccctccc ctctggacc tggcattatc cctccctatc ccggcagcct
961  gcctggaaac tccccgtcgt cccactacc cagtgtctga tccctagacc tggccccctt
1021  tcgtggtaaa acaagccagc gccactctgg tctggagtag taatcacctt gccgccccct
1081  caggggcgcc ggaagccctt tcttgctagg ctttcttagg aacagggatc aaattacacc
1141  tgtccctcac tcagtgccta atcataaagg gtcctaagaa gccgagccaa cagctcctag
1201  acttttcagc tagctgggcc actcattcct tgaaatcaag caacctgaag agtcccaccg
1261  ccaatcccac ccttaacgag tcacctccca tccctagcca gtatggcgca gaggttagac
1321  actagagggg aagagccgtc ggggaacgga acaaatggtt ttcccttttc ctttattttt
1381  tctttgaaaa acgtgtaatt tattaagggt atttgctcaa tccaaataaa acttaattta
1441  ttgaagacaa aaaaaaaaaaaa aaa

```

FIG.-2A

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FIG.-2C

1 aagcagagcc aagtttatta tgaggactat atactctaga gacctcagac aagccatctc
61 acaggaggct ttttcataaa actaggctcg gctggtagta aggaggccag tgtggaggca
121 ggcgttgagc agtgcacatc tcccactcc agccaccgtc tccacatcca tcttttattt
181 catttttcca cttggctgag ccatccagaa ccttttcaat gtataaaatg gaatatctct
241 acctcaattc ctctgcctac gactctcagt tccctgcagc tttccagtat aaccgataa
301 cagcatatgc agacttcagt tccctgcagc tccggttgcc cgtccctcac gccgggatcc tgcagccctg
361 ggaccacttt tggggccacg agcagtcctg acgccgcagt tccatacaaa ctcttcaccg
421 gcacctcag ggaccaccag aacgcaagc agcggcgcat ccgcaccacc ttcacaagcg
481 accacggcgg cctcaacgag aggtctctcg ctgagacgca ctaccctgac atctacacca
541 cgcagctcaa agagttagg atcgacctca ccgaggcgag agtccagggtg tggttccaga
601 gggaagagct agcactgaag aagcaggagc gcgcagccgc cgtgctgcc gccgccgcca
661 accgccgggc taagtctgc ctccgggaag aagtctgact cctcccgga cgacgagagc aaagaggcca
721 aaaacggctc tccgacagc actgggggcc ccgagcccaa ccccaaccg accccagct
781 agagcactga tccgacagc ggcgaggggc ccagccagc cgagagctcg ggggcggcgg
841 gtggggcaaa tggcgcggtt gaaccggca agggcggtgc ggctgcggct gctgctgcag
901 gccggggggg cccgggaggc gctgcggctg cggcgggcggg aggcctggct gcggccggag
961 ctgcagcggc tgcagcggcg cctggcccg gcccacac ctccatccca gattctctg
021 gcccgggaca aggtgggct ttatcttcgc tccaaagacc caacggtgcc aaagccgcct
081 gggggccctt cgccagcgtc tgatctgcga tctgcggcgg cggcgggcga gccgggggcc
141 tagtgaagag cagtatgttc tgggtagacc caaggctagt gtcgctgctg tcagtggctt
201 cgggctggcg agtgggcgag gcctaaaaatg atcgcgattc gaaaaaaca cgagaaaaatg
261 tttttttttt atttcaacc cactcctacc cctttcctca ccccaaca aagcaaaaa
321 acgtcgctcc aaatactcac ctgccttggg ctgcgcatag gacagggtc cacctgctgc
381 aaacaaacaa aatcttcac ctgccttggg ctgcgcatag gacagggtc cacctgctgc
441 ccaagggatg tgagcttggc cttcggggca ctctcagggt gctgtgtctg agtgacgggt
501 gtatgtctgt ctcagagaat gtgtgtctgt gggcccaagt aggtgatagg gagagacggg
561 ggaccacaaa ccaactcagt gacttcttag gaaaaaaaaaaaa

SUBSTITUTE SHEET (RULE 26)

NCSCs (2.5 days)

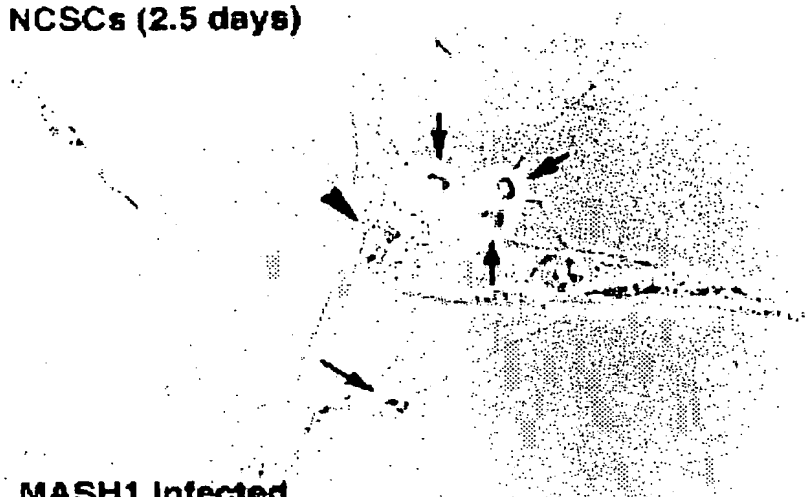


FIG._3A

MASH1 Infected

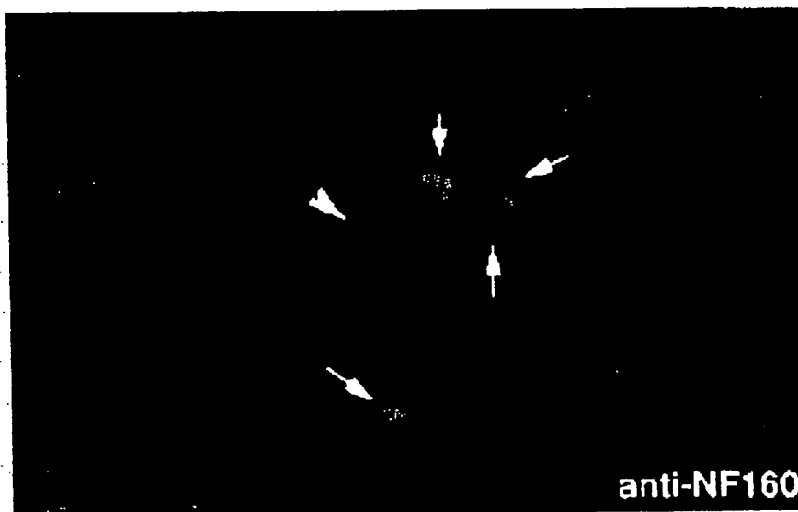


FIG._3B

anti-NF160

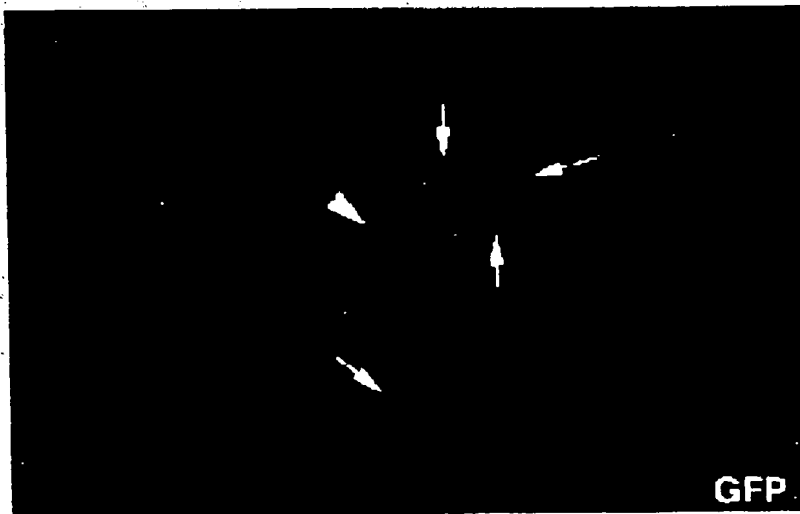


FIG._3C

GFP

NCSCs (2.5 days)

Neurogenin 1 infected

FIG._4A

FIG._4B

anti-NF160

FIG._4C

GFP

Neural crest explant (6 days)



FIG._5A

Neurogenin 1 Infected



FIG._5B



FIG._5C

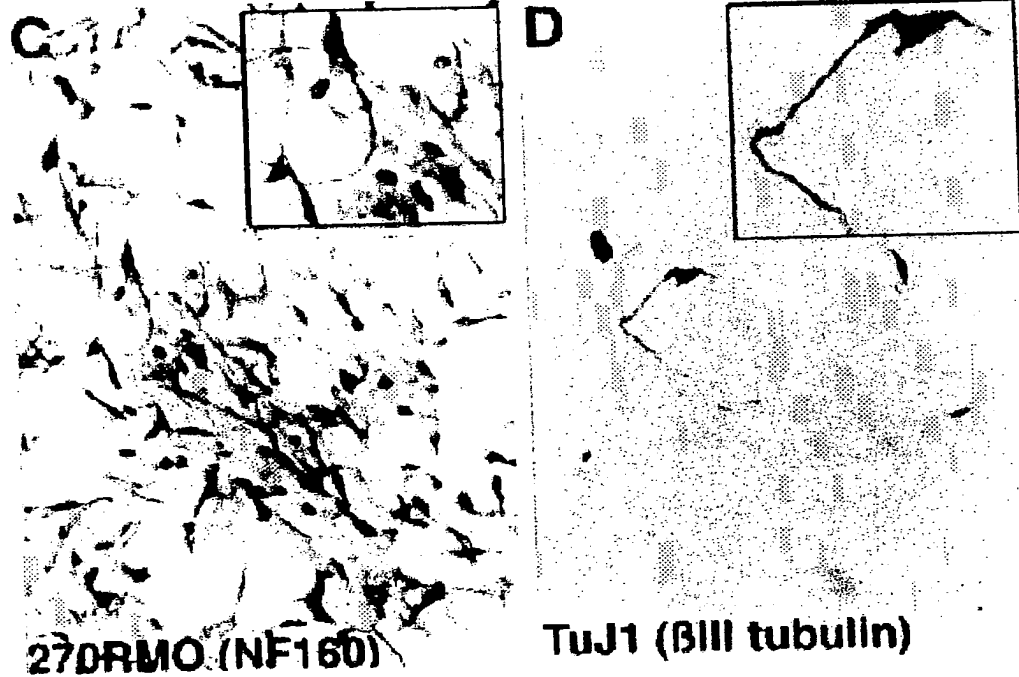
FIG._6A

FIG._6B



ngn1-myctag

3A10 (NAPA-73)



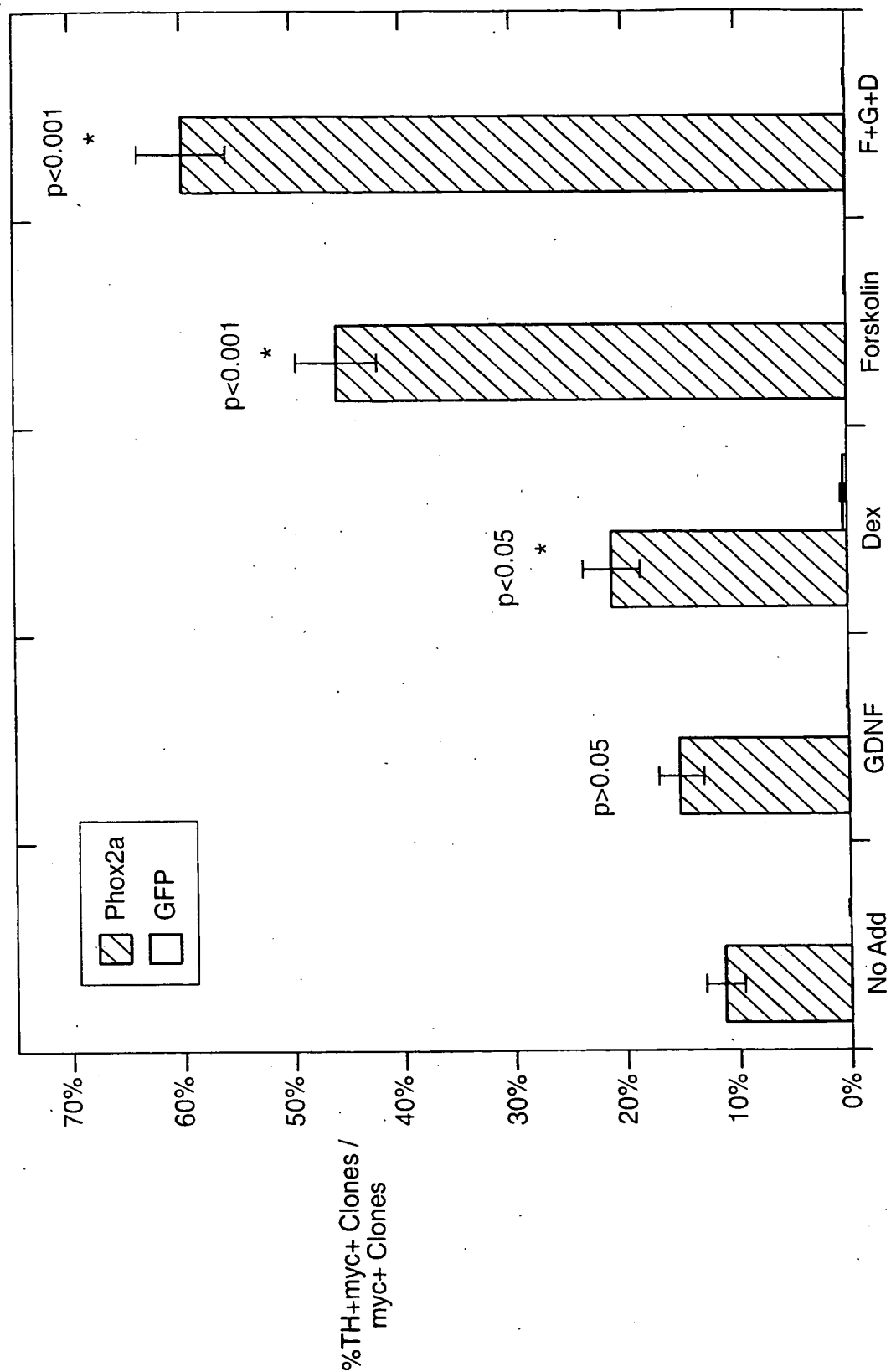
27DRMO (NF160)

TuJ1 (βIII tubulin)

FIG._6C

FIG._6D

Effect Of Different Factors On TH Induction On Phox2a Or GFP Retroviral Infected NCSCs

**FIG. 7**

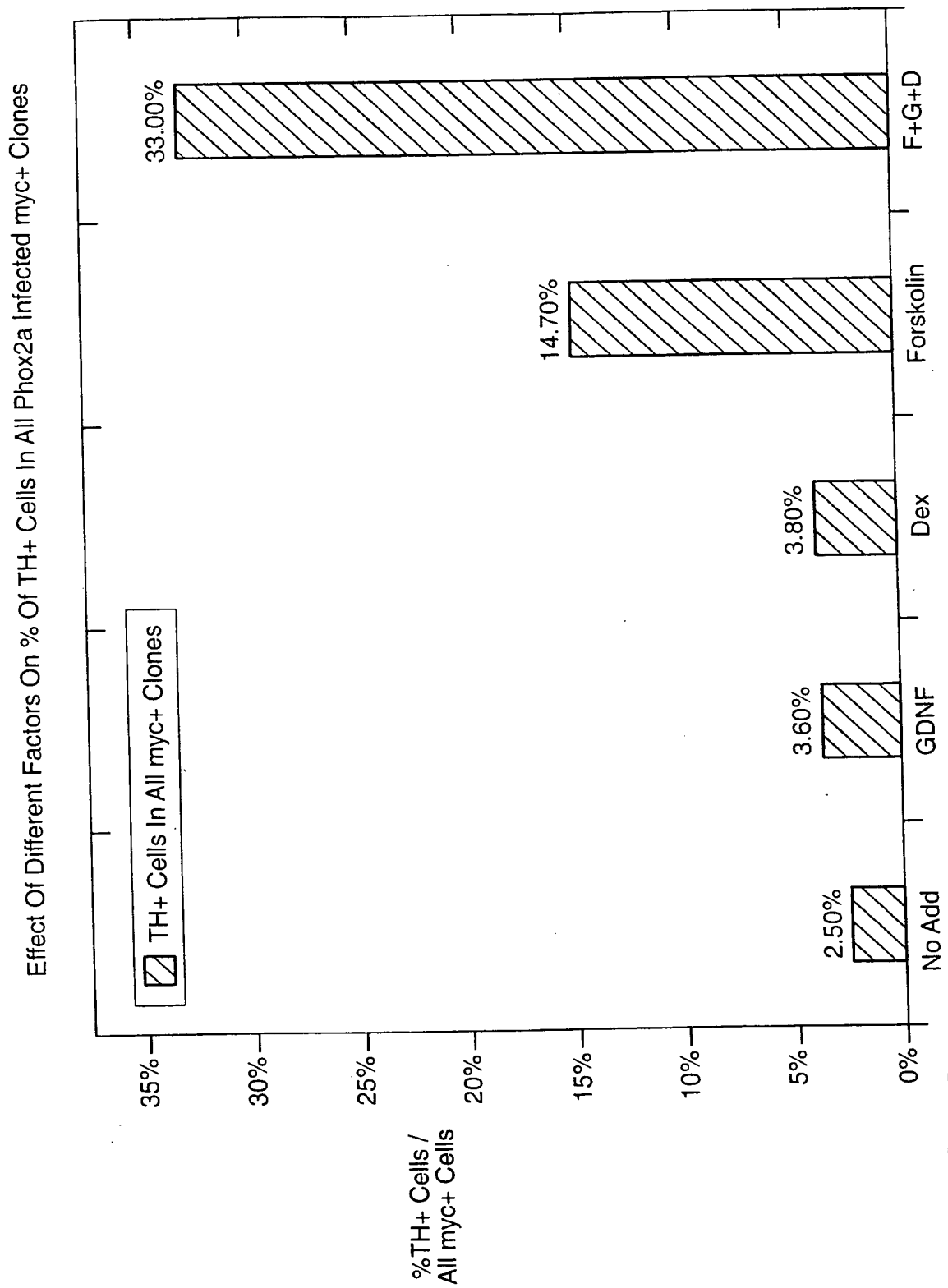
**FIG. 8**

FIG._9B

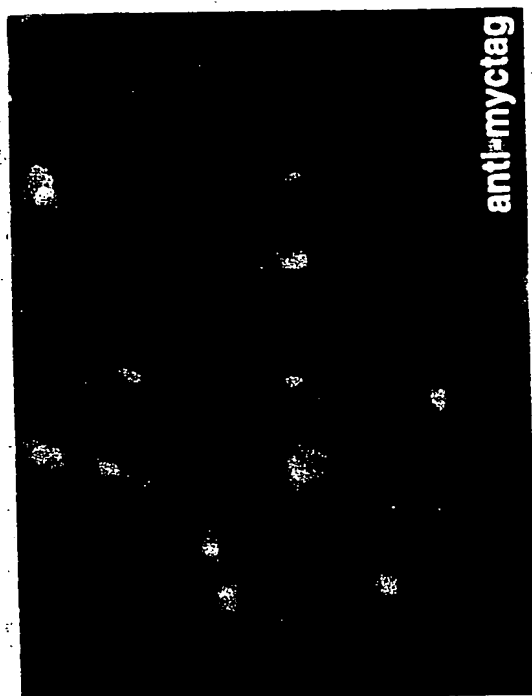


FIG._9D

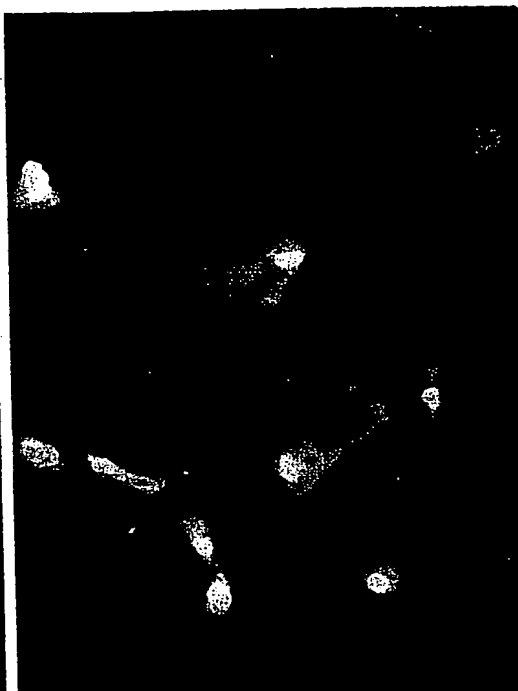


FIG._9A

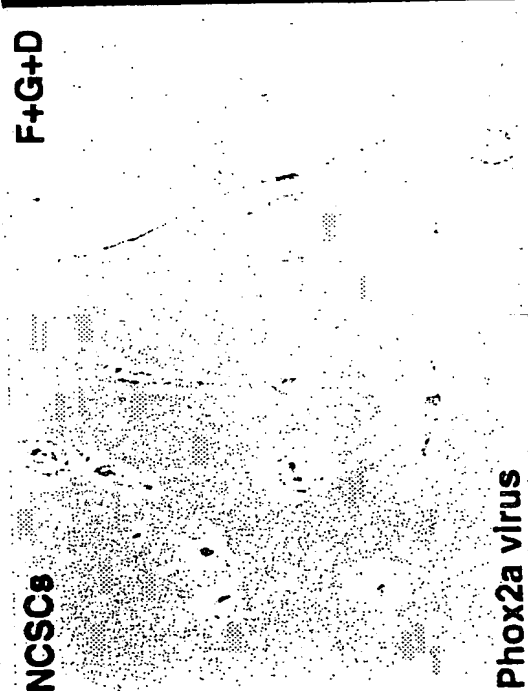
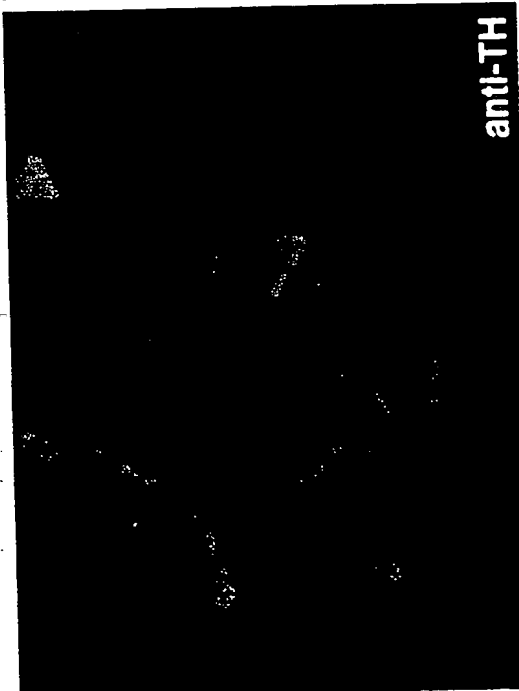


FIG._9C



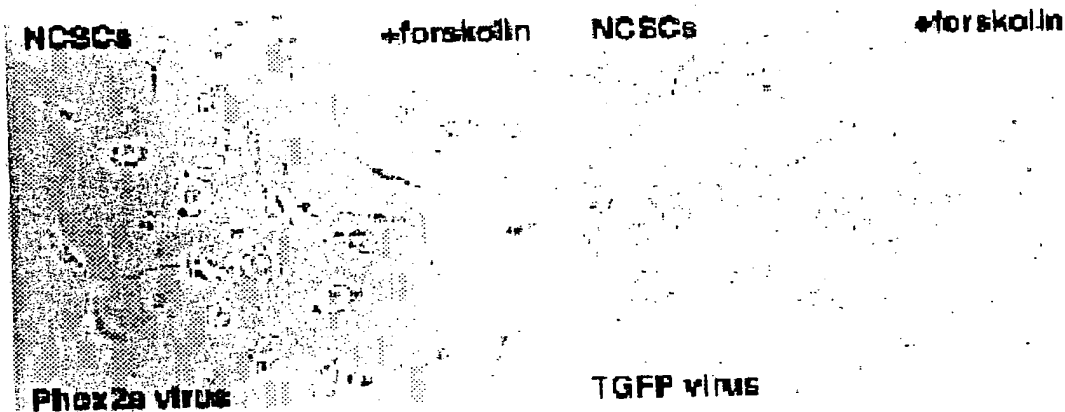


FIG._10A

FIG._10B

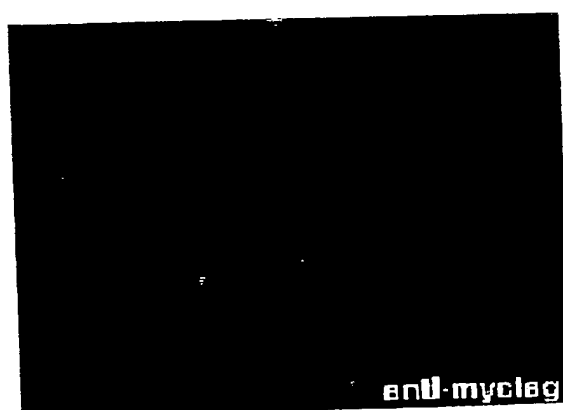


FIG._10C

FIG._10D

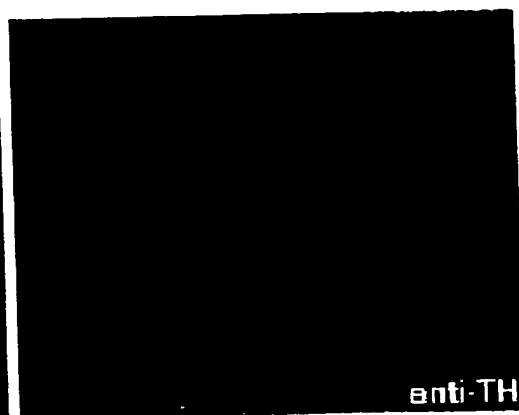
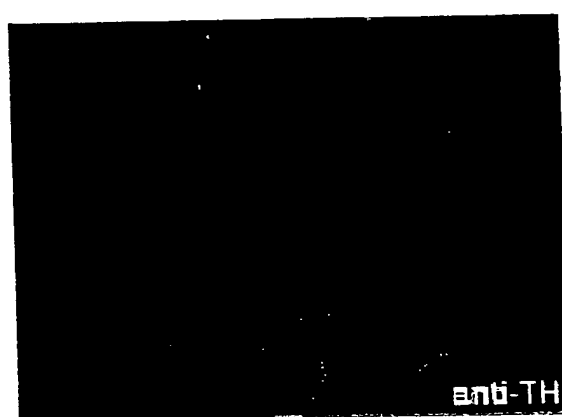


FIG._10E

FIG._10F



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 5/06, 15/85, C07K 14/475 C12N 15/66, 15/86, G01N 33/53	A3	(11) International Publication Number: WO 00/09676 (43) International Publication Date: 24 February 2000 (24.02.00)
(21) International Application Number: PCT/US99/18525 (22) International Filing Date: 13 August 1999 (13.08.99) (30) Priority Data: 60/096,630 14 August 1998 (14.08.98) US (71) Applicant: CALIFORNIA INSTITUTE OF TECHNOLOGY [US/US]; 1200 East California Boulevard, Pasadena, CA 91125 (US). (72) Inventors: ANDERSON, David, J.; 2891 Mount Curve Av- enue, Pasadena, CA 91001 (US). LO, Li-Ching; 525 West Las Tunas Drive, Arcadia, CA 91006 (US). (74) Agents: TRECARTIN, Richard, F. et al.; Flehr Hohbach Test Albritton & Herbert LLP, Suite 3400, 4 Embarcadero Center, San Francisco, CA 94111-4187 (US).		(81) Designated States: CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> (88) Date of publication of the international search report: 8 June 2000 (08.06.00)
(54) Title: METHODS OF FORMING NEURONS (57) Abstract The invention relates to novel methods of inducing non-neuronal cells to differentiate into neurons and to methods of inducing non-neuronal cells to express a neuronal subtype-specific marker.		

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INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/US 99/18525

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N5/06 C12N15/85 C07K14/475 C12N15/66 C12N15/86
G01N33/53

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 13491 A (CALIFORNIA INST OF TECHN)	1-4, 7, 8,
Y	2 April 1998 (1998-04-02)	14, 22
	page 4, line 12 -page 7, line 2; examples	10, 12,
	1-4	15-17,
	---	23, 24
	-/--	

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Y	<p>LO L ET AL., : "MASH1 activates expression of the paired homeodomain transcription factor Phox2a, and couples pan-neuronal and subtype-specific components of autonomic neuronal identity" DEVELOPMENT, vol. 125, 22 January 1998 (1998-01-22), pages 609-620, XP002130309 cited in the application abstract page 610, right-hand column -page 611, left-hand column page 614, right-hand column -page 619, left-hand column</p>	10,12, 15,16,23
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X	<p>MCCORMICK M B ET AL: "NEUROD2 AND NEUROD3: DISTINCT EXPRESSION PATTERNS AND TRANSCRIPTIONAL ACTIVATION POTENTIALS WITHIN THE NEUROD GENE FAMILY" MOLECULAR AND CELLULAR BIOLOGY,US,WASHINGTON, DC, vol. 16, no. 10, October 1996 (1996-10), page 5792-5800 XP002071058 ISSN: 0270-7306 cited in the application the whole document Fig 2</p> <p>---</p>	1,2,8, 10,11,22
X	<p>SOMMER L ET AL: "NEUROGENINS, A NOVEL FAMILY OF ATONAL-RELATED BHLH TRANSCRIPTION FACTORS, ARE PUTATIVE MAMMALIAN NEURONAL DETERMINATION GENES THAT REVEAL PROGENITOR CELL HETEROGENEITY IN THE DEVELOPING CNS AND PNS" MOLECULAR AND CELLULAR NEUROSCIENCES,US,SAN DIEGO, vol. 8, no. 4, 1996, page 221-241 XP002072615 ISSN: 1044-7431 cited in the application the whole document Fig 1</p> <p>---</p>	1
A	<p>SHIMIZU C ET AL: "MATH-2, A MAMMALIAN HELIX-LOOP-HELIX FACTOR STRUCTURALLY RELATED TO THE PRODUCT OF DROSOPHILA PRONEURAL GENE ATONAL, IS SPECIFICALLY EXPRESSED IN THE NERVOUS SYSTEM" EUROPEAN JOURNAL OF BIOCHEMISTRY,DE,BERLIN, vol. 229, no. 1, 1 April 1995 (1995-04-01), page 239-248 XP002039567 ISSN: 0014-2956 the whole document</p> <p>---</p>	1
A	<p>SOMMER L ET AL: "THE CELLULAR FUNCTION OF MASH1 IN AUTONOMIC NEUROGENESIS" NEURON,US,CAMBRIDGE, MA, vol. 15, no. 6, December 1995 (1995-12), page 1245-1258 XP002071060 cited in the application the whole document</p> <p>---</p> <p style="text-align: center;">-/--</p>	1

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Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TURNER D L ET AL: "EXPRESSION OF ACHAETE-SCUTE HOMOLOG 3 IN XENOPUS EMBRYOS CONVERTS ECTODERMAL CELLS TO A NEURAL FATE" GENES AND DEVELOPMENT, US, COLD SPRING HARBOR, NY, vol. 8, page 1434-1447 XP002071061 ISSN: 0890-9369 the whole document ---	1
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